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The Use of Oil of Citronella* for the Protection of Lambs against Blowfly Strike.

By F. G. Lennox, M.Sc., A.I.C.,† *and D. L. Hall.*†

Summary.

Turntable trapping experiments demonstrated the repellency to blowflies of oil of citronella, and insectary tests with a pure population of *Lucilia cuprina*, the Australian sheep blowfly, showed that the application of solutions of the oil to the fleece surrounding attractive areas on sheep reduced the fly oviposition on these areas.

When applied to the tail and purse of lambs at "marking" (i.e., tailing and castration), 5 or 10 per cent. concentrations of the oil in an aqueous soft-soap solution significantly reduced the proportion of animals struck during the subsequent period of healing.

1. Introduction.

A desirable way of protecting sheep against blowfly strike would be to make them unattractive to the fly by treatment with some material which will offset the attractive odour. This would appear to be a particularly suitable method for application to lambs immediately after "marking," because, until the tailing and castration wounds are healed, these areas are usually highly susceptible to bacterial infection and strike.

Theoretically, dressings may render the attractive odour ineffective by reacting chemically with the substance producing it, by masking it without chemical interaction, or by repelling the flies which would otherwise be attracted.

Essential oils which are investigated in this paper probably mask or act as true repellents rather than destroy the odoriferous putrefactive substances which attract the fly.

* Oil of citronella (Ceylon) was used throughout this investigation.

† An officer of the Division of Economic Entomology, Canberra.

2. Laboratory Testing of Citronella as a Repellent.

(i) *Comparison with Other Oils by the Turntable Method.*

An approximate classification of essential oils on the basis of fly repellency was made possible by the use of the standard glass traps and turntable designed by A. J. Nicholson (Freney, 1937). The oil under examination was placed in a small porcelain crucible which, in turn, was floated in the carrion bait, as described by Freney for the testing of mixed odours. Three oils were tested in duplicate (simultaneously) on each turntable in the field, and the repellent action was judged by comparing the number of flies caught in each of the test traps with those caught in control traps on the same turntable. The control traps contained carrion but no essential oil.

The oils are classified into three groups on the basis of qualitative tests—the relative numbers of flies in the test and control traps being judged by inspection. The efficiency of those in the first group was confirmed by re-testing.

(*a*) Highly repellent: citronella, coriander, lavender, petit grain, rosmarini, rutae.

(*b*) Slightly repellent: absinthe, bitter almond, camphor, cinnamon, cubebs, fennel, geranium, jasmine, juniper berries, lauro-cerasus, lime, parsley, tansy, valerian, verbena, ylang ylang.

(*c*) Non-repellent: anisi, augerlich, bay, bergamot, betulae, cadin, cajuput, cardamom, chenopod, copaiba, croton, macis, origanum, patchouli, sassafras.

An examination of the compositions of the most effective essential oils failed to show any correlation between repellency and the predominance of particular chemical compounds.

Since citronella is the cheapest of the oils in the highly repellent group it may have some field application and was consequently chosen for further study. Indoor repellency tests conducted by D. F. Waterhouse (unpublished) with pure cultures of *L. cuprina* also support the view that oil of citronella is a blowfly repellent.

(ii) *Preparation of Aqueous Soap Solutions of Citronella.*

Medicinal paraffin has been used as a base for oil of citronella in some of the experiments described in this paper. It penetrates into the fleece well, it is non-irritant to the skin, and, being practically non-volatile, it should liberate the dissolved essential oil only very slowly. However, experiments show that paraffin oil accumulates dust on the fleece and promotes infection, and for reasons of economy also it is not likely to find wide scale field application.

A more suitable solvent in the form of an aqueous soap solution was brought to notice by a recent publication of Albert (1939). He pointed out that oil of citronella, along with several other well-known essential oils, have their water-solubilities increased by the presence of soaps. Concentrated soap solutions dissolve relatively large amounts of oil to form transparent solutions, which retain their transparency on dilution.

Ammonium ricinoleo-sulphate with ammonia as an auxiliary solvent, potassium ricinoleate with sodium chloride auxiliary, and triethanolamine ricinoleate (basic) were all recommended as solvents for citronella. On the grounds of cost and with a view to the possible use of citronella solutions to repel sheep blowflies under field conditions, these were rejected in favour of soft soap, which, in hot concentrated solution, was found to be a satisfactory solvent.

For the preparation of 10 per cent. citronella solutions, the following proportions of the ingredients were used:—

Oil of citronella, 10 per cent. (by weight).
Soft soap, 10 per cent. (by weight).
Water, 80 per cent. (by weight).

The soft soap was dissolved in 50 parts of water by mixing thoroughly and heating almost to boiling. The citronella was added slowly* to the hot soap solution with continuous stirring. A cover was then placed over the vessel to minimize loss of oil of citronella by volatilization, and warming was continued for several minutes until the preparation became uniformly transparent and syrupy. The remainder of the water was added and mixed, and the solution was allowed to cool.

For the preparation of a 5 per cent. aqueous citronella solution the 10 per cent. solution was diluted with an equal volume of water.

The slightly cloudy appearance of the final product was also observed in soap solution of the same concentration, and, therefore, it could not be attributed to undissolved essential oil.

(iii) *Deterrent Effect of Citronella on L. cuprina Oviposition.*

The turntable method of repellency testing in the field, described previously, may give an unreliable indication of the value of a material for the protection of sheep against blowfly strike for the following reasons:—

1. Only about 2 per cent. of the flies caught in the field with carrion baits are *L. cuprina,* the most important Australian sheep blowfly.
2. Measurement of the degree to which a sample of carrion bait in a blowfly trap is made unattractive by a substance, may not give a true indication of the oviposition deterrent action of this substance.
3. Carrion bait may not exhibit the same type of fly attractiveness as is exhibited by sheep. According to Hobson's interpretation of sheep attractiveness, carrion would lack the sheep or S factor (Hobson, 1936).

These difficulties were avoided by testing the citronella oil on sheep in an insectary containing a pure fly population of *L. cuprina.* The technique, which will be described in detail by I. M. Mackerras and M. J. Mackerras (unpublished), involves making a particular area on a sheep attractive by placing in the fleece a plug of absorbent cotton soaked in a solution of indole and ammonium carbonate, and surrounding this area of fleece with a ring of the substance under investigation. Two areas, situated on the anterior and posterior regions respectively on the

* If added carelessly the solution may boil violently and froth over.

backs of the sheep, were made attractive. One was ringed with the test solution and the other with the solvent to serve as a control. The oviposition was compared at intervals on freshly impregnated plugs in each of the areas by counting the number of egg batches deposited during a 5 or 6-hour period.

A comparison between a 50 per cent. paraffin solution of citronella and the pure solvent is given in Table 1. A record was kept of the number of egg batches and the number of areas or sheep between which these batches were distributed.

TABLE 1.

Tested at the following Intervals after Treatment (Days).	No. Sheep Tested.	Paraffin.		50 per cent. Citronella in Paraffin.	
		No. Areas "Struck."*	No. Egg Batches.	No. Areas "Struck."	No. Egg Batches.
0	6	6	27	Nil	Nil
6	6	6	42	Nil	Nil
10	5	2	6	Nil	Nil
15	5	4	14	Nil	Nil

* The term "Struck" here indicates fly oviposition on the attractive areas.

These results show that the 50 per cent. citronella solution conferred excellent protection against oviposition for at least a fortnight after application.

Similar experiments were conducted with 10 per cent. citronella solutions in paraffin and aqueous soft soap. The results of these tests are given in Tables 2 and 3 respectively.

TABLE 2.

Expt. No.	Tested at the following Intervals after Treatment. (Days).	No. Sheep Tested.	Liquid Paraffin.		10 per cent. Citronella in Liquid Paraffin.	
			No. Areas "Struck."	No. Egg Batches.	No. Areas "Struck."	No. Egg Batches.
1	0	10	6	11	2	2
	4	10	7	12	0	0
	7	10	7	22	1	1
	14	10	10	47	8	17
2	0	10	10	33	0	0
	8	10	8	35	7	24

Although the dilute citronella solutions are much less effective deterrents of oviposition than 50 per cent. solutions in liquid paraffin, they do confer significant protection on the sheep for a short period after application. This applies equally to both solutions; comparison of the paraffin solution results (Table 2) with those obtained when using aqueous soap solutions (Table 3) fails to reveal any definite superiority

TABLE 3.

Expt. No.	Tested at the following Intervals after Treatment. (Days).	No. Sheep Tested.	Soap Solution.		10 per cent. Citronella in Soap Solution.	
			No. Areas "Struck."	No. Egg Batches.	No. Areas "Struck."	No. Egg Batches.
3	0	10	10	96	1	1
	2	7	7	129	6	13
	9	6	6	80	6	17
	16	6	6	55	6	40
4	0	10	10	237	9	15
	6	10	10	243	10	99
	12	10	9	49	7	30

of either group. However, a direct comparison of pure liquid paraffin with soft soap solution without citronella showed that the former prevented oviposition more effectively than the latter solvent.

As one might expect, the ratio of the daily oviposition on the test to the daily oviposition on the control groups increases with time, due to gradual loss of the citronella by evaporation. In examining the tables, the number of egg batches on the citronella treated areas should always be considered in relation to the number obtained on the same day on the untreated control areas, because the extent of oviposition will be affected by factors such as temperature, relative humidity, and the physiological state and density of the fly population, all of which vary from day to day. During this particular investigation, for example, only 11 egg batches were obtained in the control group on the first day of Experiment 1, and 243 batches on the seventh day of Experiment 4. Consequently, the number of egg batches on the treated animals in the latter case was also exceptionally high.

Thus, it appears that treatment of sheep with citronella solution can be relied on to reduce oviposition on attractive areas. But, although the number of egg batches on the treated animals will be considerably fewer than on the untreated animals, in both groups the amount of oviposition will vary with what may be described as the "oviposition potential" of the fly population at the time of testing. Also, the degree of protection against oviposition will diminish progressively with length of time after application of the repellent.

3. Protection of Lambs with Citronella Solution after Marking.

In two successive seasons the lambs in the experimental flock at Canberra have been treated with citronella solution for protection against blowfly strikes.

In the 1938 season the 72 lambs were treated in two batches. Approximately four weeks after birth the lambs were marked, and 5 per cent. citronella solution was swabbed on the wounds and the surrounding fleece of half the animals. It was spread over the inner breech area and, in the case of the wethers, the area surrounding the purse wound was also liberally dressed.

Since the citronella solution was applied to each lamb immediately after operating, before the bleeding had ceased, it was carried away from actual contact with the raw areas. Therefore, prevention of oviposition on the exposed tissues must rely solely on the protective effect of the vapour from the oil on the surrounding skin and fleece.

The lambs were yarded and examined at intervals of from 1 to 3 days after treatment, and the presence of live maggots on the raw tissues was recorded as strike. Table 4 shows the incidence of strike in treated and control groups of ewes and wethers. Paraffin oil was used as the solvent for the 5 per cent. citronella applied to the first batch, but, as the results obtained did not differ significantly from those obtained with the soap solutions used on the second batch, they are all grouped together in the table.

In an attempt to confer greater protection than in the previous season, the concentration of citronella for the 1939 experiment was increased to 10 per cent., and in order to avoid any risk of spreading blood from the raw tissues to the surrounding fleece the swabbing method of applying the essential oil was replaced by spraying. The ordinary hand-operated garden spray, fitted with a coarse nozzle, was found to be quite effective for the purpose. This time half the tails were docked at the second joint from the base (medium) as in the previous season's experiment, but the remainder were cut at the third joint (long).

The results for both seasons are presented together in Table 4.

TABLE 4.

Season.	Tail Length.	Sex.	Untreated.		Treated with Citronella.	
			No. in Group.	No. Struck.	No. in Group.	No. Struck.
1938 (5% Citronella) ..	Medium ..	Ewes ..	20	18	20	14
		Wethers ..	16	13	16	7
1939 (10% Citronella) ..	Medium ..	Ewes ..	10	6	10	2
		Wethers ..	9	5	9	1
	Long ..	Ewes ..	9	2	10	Nil
		Wethers ..	7	Nil	10	Nil

In both seasons the citronella treatment has afforded quite significant protection against strike. There appears to be no correlation in these experiments between susceptibility and the sex of an animal, but in a previous experiment, during the 1937 season, the ewe lambs were found to be more susceptible than the wethers.

It will be noted that tailing at the third instead of the second joint has considerably reduced the strike incidence in the 1939 experiment.

To indicate thė rate at which strikes occurred in both the treated and control groups, the appropriate figures have been recorded graphically in Fig. 1.

In Experiment B only the medium tail group has been considered, and in both experiments the proportion of strikes is expressed as percentage. This enables the curves for both seasons to be compared directly.

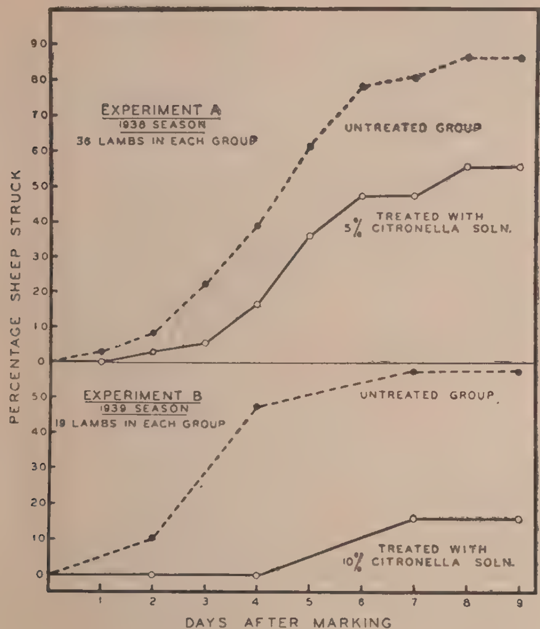

Fig. 1.—The Incidence of Strike at Various Intervals after Marking.

These curves show that, not only is the ultimate protection resulting from citronella treatment appreciable, but also that, at all stages during healing, the incidence of strike is lower amongst the treated animals.

The approximately sigmoid shape of the strike curves in Fig. 1 could be explained as follows:—The initial almost horizontal portion is due to an unattractive phase immediately after tailing. Presumably, the flies are not appreciably attracted by fresh undecomposed blood. However, by about the third day, putrefaction of the blood, serum, and other components of the wound discharge had commenced, and this marked the development of a highly attractive phase during which most of the strikes occurred. A record of the development of sepsis in tails showed that this also coincided with the highly attractive phase and, no doubt, it is partly responsible for attractiveness. By about the seventh day the scab was dry and clean and the wound no longer attractive to the fly. Therefore, most of the lambs which were not struck prior to this stage recovered without strike. This accounts for the upper flat portion of the curves.

The reduction of the incidence of strike resulting from treatment of lambs with citronella solutions is probably due to the following factors:—

(1) Oil of citronella repels flies from the wound, and may also interfere with the normal oviposition stimulus of those which alight on or in the vicinity of the wound.

(2) Through both antiseptic and stimulant action citronella oil, absorbed from the air in the vicinity of the breech, promotes the healing of wounds. Support for this view is provided by the laboratory records for 1939 which show that the proportion of wounds which healed within a week was much greater in the citronella-treated than in the control group. On the other hand, in the 1938 season when 5 per cent. citronella solutions were used, infection was equally severe in both groups.

(3) Toxicity experiments on *L. cuprina* prepupae (Lennox, 1940) showed that undiluted oil of citronella exerts some contact action and although, as applied to lambs in 5 or 10 per cent. solution, the contact toxicity will be considerably reduced, freshly emerged larvae are more susceptible than prepupae (K. C. Richardson, unpublished), and the development of strike may be prevented by the destruction of those which come into direct contact with the solution.

A reduction in the incidence of strike was also obtained by the application of a 50 per cent. paraffin solution of oil of citronella to lambs after marking at the National Field Station, "Gilruth Plains," Cunnamulla, Queensland. In a control group of 160 wethers, seven strikes were recorded, whereas, in a group of 159 wethers treated with the citronella solution, no strikes occurred. The proportion of strikes in the control group of untreated animals at Cunnamulla was very low compared with the control group strikes in the Canberra experiments, and this explains the complete absence of strike from the treated group at Cunnamulla. If this large scale experiment had been conducted during the peak of the fly wave, the number of strikes in both groups would have been considerably higher.

4. Discussion.

From a consideration of Fig. 1 it would appear that the ideal repellent preparation for application to lambs after marking would be one exhibiting maximum protection between the second and seventh days. Obviously this delayed maximum cannot be attained if the preparation is applied only on the same day as marking, since the oil is being gradually reduced in concentration by evaporation and the repellent effect will be correspondingly diminished. A close approximation to this ideal should be attained if the solution were applied at marking and again three days later, but the extra protection obtained by this method may not justify the extra handling involved.

The odour of oil of citronella can be detected on treated animals for at least fourteen days after application. Presumably it is held in the yolk and the wool fibres, and in a soap film which remains after evaporation of the water, and therefore it is only lost by very gradual evaporation.

As with aqueous soap solutions and soapy emulsions in general, the soap solutions of citronella recommended in this paper penetrate into the fleece very readily but, unfortunately, by virtue of their surface tension lowering effect on water, they also promote the wetting of the same area when sheep are exposed to rain. Thus, the presence of soap in the fleece encourages leaching out of the fly repellent, and if heavy rain falls within a few days of treating lambs with the repellent solution the protective effect of the treatment may be diminished.

Kerosene-Stockholm-tar mixtures, which are sometimes used at lamb-marking in this country, damage the fleece on the areas to which they are applied and they are difficult to remove in scouring. Citronella soap solutions on the other hand, being of a pale-yellow colour, do not stain the fleece, and they should be readily removed from the fleece in the scouring process.

Higher concentrations of oil of citronella than 10 per cent. were not tested because, in addition to rendering the treatment prohibitive on the score of cost, they could not be dissolved in aqueous solution without so increasing the soap concentration that the preparation became too viscous, and they are approaching the limit for non-irritant action on the skin of the sheep. Slight irritation has been recorded, for example, with 20 per cent. citronella solutions in liquid paraffin.

5. Conclusion.

In conclusion, it must be emphasized that the treatment of lambs with citronella solutions, or for that matter with any other blowfly repellent, is not likely to give complete protection against strike unless either the severity of the strike wave at the time of tailing is very low or the treatment is applied in conjunction with some other protective measure, such as tailing at the third joint.

However, on the evidence collected in these preliminary experiments, the treatment would appear to reduce the incidence of strike—chiefly through its fly repellent action—and the circumstances governing its successful application in the field will only be determined by large-scale trials.

6. Acknowledgments.

The authors are indebted to Drs. I. M. and M. J. Mackerras for carrying out the oviposition experiments, and also to Mr. D. J. Lee for co-operation in the performance of field repellent tests.

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Skeleton Weed, *Chondrilla juncea* L.

Experiments with Weed-Killers II.

By A. B. Cashmore, and K. G. Carn.†*

Summary.

The field experiments described in this paper were designed to test chemical weed-killers as a means of controlling skeleton weed. They were carried out in the Wagga district of New South Wales in the 1937 to 1939 period, and form part of a general scheme of review of available weed-killing materials.

The results of the experiments, which involved twenty treatments, showed that sodium chlorate, certain arsenic compounds, and a proprietary chlorate weed-killer, caused reductions in weed plant numbers fourteen to seventeen months after application. The November and December applications of sodium chlorate were more effective than those applied earlier in the 1937 season.

Only the sodium chlorate (200 lb. or more per acre) treatments affected subsequent soil productivity as measured by the establishment of the wheat crop and the yield of grain on weed-free land. The degree of control of the weed more or less paralleled this residual soil-sterilizing effect.

Certain treatments which were not effective in reducing the actual skeleton weed population did lead to increased yields of wheat grain on infested land.

The work is being continued.

1. Introduction.

In an earlier paper (1) the authors have reviewed the literature on the introduction of skeleton weed and its distribution in the Riverina district of New South Wales, and have discussed the importance of the plant as a weed in that area. Experiments with weed-killers on skeleton weed were conducted near Wagga Wagga during the 1935-36 season, and the results of this work were detailed also. These indicated that, at the rates of application used, only common salt and sodium chlorate were effective in leading to reductions of skeleton weed plant numbers, and that these materials in effective doses also led to a reduction in subsequent soil productivity. It was suggested that the effects were due to a measure of soil sterilization, that absorption through the roots was responsible for their effectiveness rather than a direct killing effect as foliage sprays, and that heavier rates of application of chlorates and other chemicals would be required to obtain satisfactory control of the weed. The experimental review of available weed-killing chemicals in relation to skeleton weed control has been continued. The results obtained in the 1937 to 1939 period are reported in this paper.

2. Experimental Procedure.

As in the previous work, two areas of typical wheat-growing land were selected for use in the experiments. The first, on a privately-owned farm near Wagga Wagga, had not been cropped for several years but was found to be infested, at the time of application of the treatments, to the extent of thirteen skeleton weed plants per square link. The actual mean figure obtained was 12.96 ± 2.05 plants, and the ratio of treatment to error mean square was 1.1 where F at .01 is 2.29. In making

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the counts no attempt was made to discriminate between established plants and seedlings, nor was any estimate of ground cover obtained. This land, more sandy than the red-brown earths common to the district, was considered to be heavily and uniformly infested. The second area, on which the residual effect of the chemical treatments was tested, is located at the Wagga Experiment Farm, Bomen. The soil is a medium loam and was free of skeleton weed. Similar treatments were applied at both centres, the experimental design including twenty treatments distributed within four randomized blocks. Individual plots measured 50.7 by 4 yards. These were so arranged that, in harvesting the wheat crop sown on the treated areas in 1938, one cut of the harvester along the centre of each plot removed the grain from an area of 1/60 acre.

The following treatments were applied in the experiments:—

Treatment.	Lb. per Acre.	Month of Treatment.
1. Control	..	..
2. Sodium chlorate	200	September
3. ,, ,, ...	200	October
4. ,, ,, ...	200	November
5. ,, ,, ...	200	December
6. ,, ,, ...	100	October
.. acetate (2)	10	
7. ,, chlorate	200	,,
.. acetate	20	
8. ,, chlorate	400	..
9. Weedex	200	..
10. Atlacide	200	..
11. Borax (3)	200	..
12. ,, ...	400	,,
13. ,, ...	200	,,
Sodium chlorate	30	
14. Soda alum	200	..
15. ,, ,, ...	400	..
16. Arsenic pentoxide	200	..
17. ,, trioxide*	50	..
18. Hardy's Weed-killer II.	20 gallons	..
19. B.A.C.	200	..
20. King's method—Ammonium carbonate	600	..

* Applied as acid sodium arsenite, 1 per cent acid.

Except with borax, all treatments were applied in solution at the rate of 200 gallons per acre. With borax, water at the rate of 400 gallons per acre was used to dissolve 200 lb. and 800 gallons for 400 lb. Treatments 14 and 15 (soda alum) were applied as heated solutions as it was necessary to boil the solution to dissolve the chemical. Treatment 2 was given on 21st September, treatment 4 on 9th November, and treatment 5 on 4th December, 1937. The October treatments were all applied, in both series of plots, during the period 7th to 15th October, except treatment 20 which was sprayed on the weed-infested land on 26th October, and on the non-infested plots on 30th October. Invariably the days chosen for carrying out the spraying work were warm and sunny. It was planned to measure the effects of treatments by means of periodic plant counts and by the yields of wheat grain obtained from the plots when cropped during the following (1938) season.

3. Results.

(a) The Effect of Weed-killers on Skeleton Weed.

To measure the immediate effects of the treatments applied, plant counts were made on 16th February, 1938. In making the counts, 10 square link samples per plot were taken and the analysis of variance applied to the means. The experimental area was ploughed on 24th February and was sown to wheat (60 lb. seed and 1 cwt. superphosphate per acre) on 9th May. Plant counts were again made on 27th July to determine the numbers of skeleton weed plants present at that time and the effects of treatments on the early establishment of wheat. The wheat plots were harvested on 25th November, and final plant counts were made on 14th February, 1939. The results are shown in Table 1.

Table 1.—Showing Results of Plant Counts and Yields of Wheat Grain on Weed-killer Plots near Wagga Wagga, 1938-39.

Plants per square link and bushels per acre.

Treatment.	16th Feb., 1938.	27th July, 1938.		14th Feb., 1939.	25th Nov., 1938.
	Weed.	Weed.	Wheat.	Weed.	Wheat Yield. Bushels per Acre.
1. Control	1·70	7·0	2·6	1·20	5·1
2. Sodium chlorate .. 200 lb. Sept.	1·00	0·3	2·9	0·35	12·4
3. ,, ,, .. 200 ,, Oct.	0·55	1·0	3·3	0·43	12·9
4. ,, ,, .. 200 ,, Nov.	0·03	0·1	2·1	0·13	6·6
5. ,, ,, .. 200 ,, Dec.	0	0·7	2·5	0·08	9·5
6. ,, ,, .. 100 ,, Oct. ,, acetate .. 10 ,,	1·25	3·7	2·9	0·83	13·8
7. ,, chlorate .. 200 ,, ,, ,, acetate .. 20 ,,	0·30	1·6	3·2	0·43	14·6
8. ,, chlorate .. 400 ,, ,,	0·15	0·2	2·1	0·13	3·6
9. Weedex .. .. 200 ,, ,,	1·38	3·1	3·9	0·75	18·4
10. Atlacide .. .. 200 ,, ,,	0·65	3·1	3·7	0·45	19·5
11. Borax .. .. 200 ,, ,,	1·93	6·7	3·4	0·88	6·8
12. ,, ... 400 ,, ,,	1·30	5·7	3·2	0·90	6·1
13. ,, ... 200 ,, ,, Sodium chlorate .. 30 ,,	2·18	8·6	3·2	0·78	10·0
14. Soda alum .. .. 200 ,, ,,	1·48	7·2	3·2	1·43	6·3
15. ,, ,, ... 400 ,, ,,	1·98	6·3	3·8	1·23	4·1
16. Arsenic pentoxide .. 200 ,, ,,	0·20	0·8	3·7	0·50	15·8
17. ,, trioxide .. 50 ,, ,,	1·15	4·5	3·4	0·68	11·9
18. Hardy's Weed-killer .. 20 gal. ,,	0·48	0·8	3·4	0·35	19·8
19. B.A.C. .. .. 200 lb. ,,	1·08	3·2	3·4	0·78	16·4
20. Ammonium carbonate 600 ,, ,,	2·03	5·9	3·2	1·20	10·0
General mean	1·04	3·5	3·1	0·67	11·2
Standard error	0·27	1·12	0·33	0·16	1·86
Standard error—per cent.	25·52	32·0	10·4	23·50	16·68
Ratio $\frac{\text{Treatment}}{\text{Error}}$ mean squares ..	7·2	6·1	2·51	6·23	7·67
F. at ·01	2·29	2·29	2·29	2·29	2·29

Reference to Table 1 shows that sodium chlorate sprayed at the rate of 200 lb. per acre in November or December or at the rate of 400 lb. per acre in October was the most effective chemical of those used, as

measured by the relative scarcity of weed plants on the treated areas at each time of counting. At the first count after spraying (February, 1938) five of the eight treatments which caused a significant reduction in weed plant numbers involved the use of sodium chlorate; arsenic, as arsenic pentoxide and as Hardy's Weed-killer, was effective, and Atlacide, a chlorate weed-killer, also reduced the weed plant population significantly. Very few seedlings were present. The figures refer essentially to established weed plants, the density limit of which is approximately two per square link.

Table 1 shows that in the July following spraying the weed population on eleven treatments was significantly less than on the control. In addition to those shown to be effective in the February count, treatments 2 (sodium chlorate in September), 9 (Weedex), and 19 (B.A.C.) showed significant effects. These were not confirmed for treatments 9 and 19 in the final count made in February, 1939, and the differences noted in July must be attributed to sampling error and possibly to reduced establishment of skeleton weed seedlings on the plots of those treatments. The order of effectiveness of treatments was still essentially the same as that noted in February, 1938, and this order was confirmed at the final count in February, 1939. At this stage, sixteen months after the main spraying period, six of the first seven places were taken by sodium chlorate treatments (treatments 5, 4, 8, 2, 3, and 7, in that order), treatment 18 (Hardy's Weed-killer) was equal fourth, and Atlacide, arsenic pentoxide, and acid sodium arsenite showed significant effects on weed plant numbers.

The figures for established wheat plants in July, 1938, (Table 1) do not reveal any reductions in rate of establishment due to the chemical treatments.

The mean wheat yields from the plots are shown in Table 1. From the point of view of this work two factors contribute particularly to the comparative yields; firstly, the effect of the competition of the established skeleton weed plants on the wheat crop and the relative modification of this effect by the weed-killers applied in the previous spring, and secondly, the residual effect of the weed-killers themselves on soil productivity. The figures show that the borax and soda alum treatments and King's method with lump ammonia had no effect on the yield of the subsequent wheat crop on skeleton weed-infested ground. The most effective chlorate treatments (4, 5, 8) as measured by plant counts had no effect on subsequent yields, but here it is suggested that the soil-sterilizing effect of these treatments balanced their effect in controlling the competing weed plants (see Discussion below). The less effective chlorate treatments 2, 3, 7, 6 (in terms of weed plant numbers), arsenic pentoxide, acid sodium arsenite, and B.A.C. Weed-killer all led to significant increases in the yield of wheat, while the highest yields were obtained from the areas treated with Hardy's Weed-killer, Atlacide, and Weedex. The discussion of these results will be undertaken below, following upon a consideration of the effects of the chemical treatments on the productivity of non-infested land.

(b) The Residual Effect of Weed-killers on Soil Productivity.

The area of weed-free land was ploughed on 30th August, 1937, prior to the application of treatments, and was sown on 10th May to wheat (60 lb. seed and 1 cwt. superphosphate per acre). Counts were

made on 27th July, 1938, to determine the effect of the treatments on the establishment of the wheat plants, and the plots were harvested on 23rd November. The results of the plant counts and the yield figures are presented in Table 2.

TABLE 2.—SHOWING THE EFFECT OF WEED-KILLERS ON THE SUBSEQUENT ESTABLISHMENT OF WHEAT AND ON SOIL PRODUCTIVITY.

Treatment.		Wheat Plants per square link, 27th July.	Mean Yield. Bushels per Acre.
1. Control ..		3·2	28·0
2. Sodium chlorate ..	200 lb. Sept.	1·9	14·9
3. ,, ,, ..	200 ,, Oct.	2·4	14·5
4. ,, ,, ..	200 ,, Nov.	1·0	3·8
5. ,, ,, ..	200 ,, Dec.	1·1	3·1
6. ,, ,, .. ,, acetate ..	100 ,, Oct. 10	3·4	29·0
7. ,, chlorate .. ,, acetate ..	200 ,, ,, 20	2.1	15·4
8. ,, chlorate ..	400 ,, ,,	0·5	1·1
9. Weedex ..	200 ,, ,,	3·3	30·3
10. Atlacide ..	200 ,, ,,	3·4	28·1
11. Borax ..	200 ,, ,,	3·0	27·6
12. ,, ..	400 ,, ,,	3·1	24·5
13. ,, .. Sodium chlorate ..	200 ,, ,, 30	3·2	30·6
14. Soda alum ..	200 ,, ,,	3·6	26·8
15. ,, ,, ..	400 ,, ,,	3·6	27·9
16. Arsenic pentoxide ..	200 ,, ,,	3·4	27·8
17. ,, trioxide ..	50 ,, ,,	3·0	28·8
18. Hardy's Weed-killer ..	20 gal. ,,	3·9	30·1
19. B.A.C. ..	200 lb. ,,	3·7	31·0
20. Ammonium carbonate ..	600 ,, ,,	3·5	28·5
General mean ..		2·8	22·6
Standard error ..		0·21	1·42
Standard error—per cent.		7·6	6·3
Ratio $\frac{\text{Treatment}}{\text{Error}}$ mean squares ..		21·3	49·6
F. at ·01 ...		2·29	2·29

Reference to Table 2 shows that definite reductions in the rate of establishment of wheat seedlings, as measured by plant counts in July, occurred on treatments 8, 4, 5, 2, 7, and 3, in that order; that is, on all sodium chlorate treatments where 200 lb. or more per acre of the chemical was used. A similar result was obtained in earlier work (1). No other treatment affected the establishment of the wheat crop. The same six chlorate treatments gave significant reductions in subsequent crop yield also. The figures for grain yield (Table 2) show that sodium chlorate at the rate of 400 lb. per acre applied in October or of 200 lb. in November or December almost prevented the growth of wheat as a grain crop, and that sodium chlorate at the rate of 200 lb. per acre applied in October (treatments 2, 3, and 7) halved the yield of the crop. No other treatment affected subsequent soil productivity as measured by yields of wheat grain.

The explanation of the less deleterious effect on soils of sodium chlorate applied before the 15th October, as compared with later applications, is to be found in the rainfall received at the Wagga Experiment

Farm in 1937, and the leaching which presumably occurred during heavy falls. The rainfall figures recorded are—September, 1.63 inches; October, 2.99 inches; November, 1.03 inches. It has been suggested (3) that, in the field, the sterilizing effect of sodium chlorate is bound up with its presence and that this chemical is removed from the soil by rainfall and leaching. The heavy falls received late in October at Wagga Wagga probably explain the similar effects of the September and early October chlorate applications and the more severe but similar effects of the November and December treatments on the subsequent wheat crop.

4. Discussion.

The results of the experiments reported in this paper show that ten of the twenty chemical treatments applied to skeleton weed had a permanent effect over the test period in reducing the density of the weed population on the experimental area. Of these, six involved the use of sodium chlorate, one was a commercial chlorate weed-killer, and three were arsenical treatments. Hardy's Weed-killer used at the rate of 20 gallons per acre (equivalent to approximately 320 lb. per acre) was the heaviest arsenic application employed and was the fourth most effective treatment. The most effective treatments, on a weed-survival basis, were sodium chlorate used at the rate of 400 lb. per acre in October or 200 lb. per acre in November or December. Heavy rains in the latter half of October are considered to be responsible for the reduced effect of 200 lb. per acre applications of chlorate in September and early October in that season. There is no evidence to support the view that the addition of sodium acetate to sodium chlorate solutions increases their effectiveness as weed-killing sprays. Arsenic pentoxide at the rate of 200 lb. per acre gave a useful measure of control, and acid sodium arsenite, applied at a rate equivalent to 50 lb. of arsenic trioxide per acre, also reduced the weed population significantly.

Sodium chlorate had an injurious effect on the establishment of wheat when applied at the rate of 200 lb. or 400 lb. per acre to the soil some five to eight months before sowing the crop. The greater depressing effect of the treatments applied after October as compared with earlier applications is explained by the heavy rainfall and consequent leaching which occurred in the latter half of October. This effect of the chlorate treatments on the establishment of the wheat is also shown in the yields obtained at harvest. These results show that sodium chlorate applied in the 1937 season in September and October reduced the subsequent grain crop by 50 per cent., while the later applications and the heavy (400 lb. per acre) application in October practically sterilized the soil for wheat in 1938. Sodium chlorate at the rate of 100 lb. per acre in October had no effect on subsequent soil productivity, nor did any of the remaining treatments, on non-infested land, affect the yield when compared with the untreated area.

The yields of grain obtained on the skeleton weed-infested plots reflect—(1) the measure of control exerted by the chemicals in killing established weed plants, and (2) the residual effect of the chemicals themselves on soil productivity. The most effective treatments, as determined by plant counts, were the sodium chlorate treatments causing the greatest reduction in soil productivity on weed-free land. These had no effect on wheat yields on the infested land and it must be concluded

that the effect of control exerted by them in removing the competition of the established weed plants was offset by the residual effect of the chemical on the soil. This sterilizing effect was not so marked on the sandy-infested land as on the heavier weed-free loam soil. The less effective chlorate treatments (on a weed plant count basis), arsenic pentoxide, acid sodium arsenite, B.A.C. Weed-killer, Hardy's Weed-killer, Atlacide, and Weedex significantly increased the yield of wheat in competition with skeleton weed. These treatments, with the exception of those involving the use of sodium chlorate, had no effect on soil productivity on clean land, and B.A.C. and Weedex had no effect on weed persistence. The explanation of the increased yields obtained following the use of B.A.C. and Weedex on infested country must be sought in a temporary weed-removal-fallowing-effect during the summer prior to sowing the crop. All treatments successfully killed the subaerial growth of the weeds following application, and these may have weakened the plants sufficiently to prevent them absorbing nutrients and moisture from the soil at the normal rate during the pre-sowing period, while not being sufficiently drastic in effect to remove them completely. There is reason to believe that the main effect of skeleton weed on wheat is exerted through the absorption of nitrates and possibly moisture from the soil into which the wheat is sown (4). The treatments in which borax, soda alum, and ammonium carbonate were used had no effect on weed numbers, on soil fertility, or on the yield of wheat on weed-infested land.

The results show that the most efficient weed-killers of those used contain the chlorate ion or arsenic as the active agent. The relative effectiveness of the materials available probably depends entirely on the amount of active material present, and the use in practice of any weed-killer will depend upon the cost per unit of the active agent and upon considerations of the effects of the chemicals on subsequent soil productivity and safety in use.

5. Acknowledgments.

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A Field Trial Comparing Phenothiazine, Tetrachlorethylene Emulsion, and Copper Sulphate and Nicotine Sulphate Mixture for the Treatment of Trichostrongylosis.

By Hugh McL. Gordon, B.V.Sc. and L. K. Whitten, B.V.Sc.†*

Summary.

1. Phenothiazine in doses of 15 grammes for weaners about five months old and weighing about 20 kilogrammes was highly efficient against *Trichostrongylus* spp. and showed encouraging results against *Oe. venulosum* and *C. ovina.*

2. Tetrachlorethylene in doses of 3.5 ml. administered in an emulsion following a dose of copper sulphate was also highly effective against *Trichostrongylus* spp.

The after-effects observed following the use of this drug may be of very serious significance when large numbers of sheep are being treated and held in yards and unless a good deal of attention is given to those individuals which become semi-comatose and are unable to rise. Under crowded conditions in yards a great many sheep are likely to be suffocated by other sheep falling on them.

3. The mixture of copper sulphate and nicotine sulphate usually prescribed against *Trichostrongylus* spp. gave very poor results. The possible reasons for this are briefly discussed and further studies on suggested reasons are indicated.

Introduction.

The search for an anthelmintic possessing a high degree of efficiency against *Trichostrongylus* spp. in sheep has been one of the chief objects of investigation by the parasitology section of the McMaster Laboratory. Gordon (1935) showed that a mixture of copper sulphate and nicotine sulphate possessed a reasonably satisfactory degree of efficiency against these parasites. McEwen (1935) in England recorded a similar finding as a result of field trials. Gordon and Clunies Ross (1936) showed that sheep dosed daily with 4,000 *Trichostrongylus* spp. larvae, and treated at intervals of three weeks with a mixture of copper sulphate and nicotine sulphate, were protected against trichostrongylosis. In this experiment, treatment at similar intervals with tetrachlorethylene, following a preliminary dose of copper sulphate, also protected against trichostrongylosis. Further studies by one of us (Gordon—unpublished) have shown that injection of tetrachlorethylene, alone, mixed with liquid paraffin, or in an emulsion, into the abomasum is followed by marked reduction in the number of *Trichostrongylus* spp. (chiefly *T. colubriformis* in the duodenum).

Ortlepp and Mönnig (1936) found that tetrachlorethylene administered after copper sulphate was a highly efficient treatment against *Gaigeria pachyscelis* and was also effective against *Haemonchus*

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contortus, Trichostrongylus spp. and *Oesophagostomum columbianum.* These authors state that the untoward effects of this drug can be largely overcome by its use in an emulsified form.

Preliminary studies (Gordon, 1939) showed that phenothiazine was highly efficient against *Trichostrongylus* spp.

The Trial.

(1) *Sheep Used.*

These were weaners (approximately five months old) forming portion of a mob of 200 from the "tail" of the weaner flock. At the time of the trial these weaners were in poor condition, some showed evidence of recent scouring and a few showed intermittent scouring still present. It is thought that the general condition of these sheep was gradually improving at the time the trial was carried out.

(2) *Conduct of the Trial.*

One hundred sheep were marked by attaching numbered metal discs. Faecal samples were collected from these sheep on three occasions prior to treatment. Four groups were selected, using the first series of egg counts as a basis for selection of sheep with comparable degrees of infestation. Faecal cultures, as well as egg counts, were made on each occasion, since the sheep harboured *Oesophagostomum venulosum* and *Chabertia ovina* in addition to *Trichostrongylus* spp. Faecal samples were collected on two occasions after treatment. Anthelmintic efficiency was based on the degree of reduction in egg count, expressed as percentage reduction, using the averages of three preliminary counts and of two post-treatment counts.

Faecal samples were collected on 16th February, 1940, 18th February, 1940, 23rd February, 1940, 28th February, 1940, and 1st March, 1940. Treatment was carried out on 23rd February, 1940. The sheep were in the yards for about four hours before treatment, and returned to pasture about two hours after treatment.

(3) *Treatments Used.*

(*a*) *Phenothiazine.*—A dose of 15 g. per head was administered by shaking up the powdered Thiox in about 60 ml. water. (The weaners were of an average weight of about 20 kg. At the previously used rate of 0.6 g. Thiox per kg. body weight these sheep would have been given a dose of 12 g. per head.)

(*b*) *Tetrachlorethylene in an Emulsion following Copper Sulphate Solution.*—A dose of 3.5 ml. tetrachlorethylene in 20 ml. of an emulsion (water, liquid paraffin, oleic acid, and triethanolamine—Swales [1939]) was used following a dose of 3 ml. 10 per cent. copper sulphate solution.

(*c*) *Copper Sulphate and Nicotine Sulphate.*—A dose of 30 ml. of a solution containing 2 per cent. copper sulphate and 2 per cent. nicotine sulphate was used.

(4) *Results of Treatment.*

1. *Sheep.*

(*a*) *Phenothiazine.*—No ill-effects were observed. The degree of staining by urine was extremely small. Staining of the wool around

the mouth by spilling of the dose during administration was noted. This could almost certainly be avoided by the use of a satisfactory suspension of the drug and suitable drenching funnel.

(*b*) *Tetrachlorethylene.*—A large proportion of the sheep dosed showed after-effects, often within a few minutes. These took the usual form observed following the use of this drug, namely, staggering gait, raising the head and staggering backwards, inco-ordination and, in severe cases, prostration. In those sheep in which prostration occurred, bloating of the rumen was marked. Two of the 25 sheep treated showed very severe effects and were unable to walk away from the yards about two and a half hours after treatment. These sheep recovered and were apparently normal by the following morning.

(*c*) *Copper Sulphate and Nicotine Sulphate.*— A few sheep showed transitory inco-ordination and staggering gait soon after treatment.

2. *Parasites.*

(*a*) *Trichostrongylus* spp.—It should be noted that these sheep carried varying degrees of infestation (chiefly light) with *Ostertagia* spp. and that in assessing the efficiency of treatment the latter parasites are included with *Trichostrongylus* spp.

Results of treatment are shown in Tables 1 and 2. Figures represent percentage reduction in egg count (number of *Trichostrongylus* spp. and *Ostertagia* spp. eggs per gram of faeces) for the individuals treated.

TABLE I.—PERCENTAGE REDUCTION IN EGGS OF *Trichostrongylus* SPP. AND *Ostertagia* SPP. PER GRAM OF FAECES.

Phenothiazine.	Tetrachlorethylene Emulsion after $CuSO_4$.	Copper Sulphate and Nicotine Sulphate.
95	95	50
87	98	0
94	96	0
75	12	86
62	25	0
92	95	0
93	0	0
50	98	0
59	62	0
36	94	77
90	0	0
90	95	25
87	0	57
98	78	80
87	94	56
91	98	50
81	85	0
94	83	20
90	95	50
85	80	30
95	88	70
97	84	53
95	88	72
..	98	30
..	85	..

TABLE 2.—SUMMARY OF RESULTS SHOWN IN TABLE 1.

Reduction in Egg Count.	Phenothiazine.	Tetrachlorethylene Emulsion following $CuSO_4$.	Copper Sulphate and Nicotine Sulphate.
Egg count reduced by 90 % or more	13/23 = 56·5 %	11/25 = 44 %	..
Egg count reduced by 80 % or more	18/23 = 78·2 %	18/25 = 72 %	2/24 = 8·3 %
Egg count reduced by 70 % or more	19/23 = 82·6 %	19/25 = 76 %	5/24 = 20·5 %
Egg count reduced by 60 % or more	20/23 = 86·9 %	20/25 = 80 %	5/24 = 20·5 %
Egg count reduced by 50 % or less	2/23 = 8·6 %	5/25 = 20 %	16/24 = 66·6 %

It is seen that phenothiazine and tetrachlorethylene emulsion following copper sulphate were highly efficient. There is little to choose between the two treatments, but with phenothiazine there were fewer cases in which the egg count was reduced by 50 per cent. or less. In the tetrachlorethylene group, there were three cases in which there was no reduction in egg count, and it is suggested that in these the copper sulphate failed to stimulate the oesophageal groove reflex and the tetrachlorethylene emulsion was not swallowed direct into the abomasum. Results in the copper sulphate and nicotine sulphate group are very poor, only 20.5 per cent. of sheep showing a satisfactory degree of efficiency (70 per cent. or greater reduction in egg count).

The reason for such poor efficiency of copper sulphate and nicotine sulphate mixture is not clear, but two possibilities suggest themselves—firstly, that the efficiency of this mixture is generally poorer under field conditions than under experimental conditions at the laboratory; secondly—and the large proportion of cases in which treatment showed an absolute lack of efficiency support the contention—the method of administration did not lead to the maximum stimulating effect on the oesophageal groove reflex, with the result that the mixture was only swallowed into the abomasum in a small proportion of sheep. It is seen that in the tetrachlorethylene group copper sulphate appeared to bring about effective reflex closure of the oesophageal groove in all but 3/25 or 12 per cent. of cases, but it should be noted that in this group the copper sulphate was administered about five seconds before the tetrachlorethylene emulsion, while in the copper sulphate, nicotine sulphate group the two drugs were administered simultaneously. It appears that some attention should be given to this point with the object of determining whether more effective swallowing into the abomasum of a drug subsequently administered may be attained by allowing a very short interval to elapse after the administration of copper sulphate. Mönnig (1937) has pointed out that, in order to stimulate the closure of the oesophageal groove in sheep in poor condition, it is necessary to precede the dose of copper sulphate and nicotine sulphate with 2.5 ml. of a 10 per cent. solution of copper sulphate.

(*b*) *Large Bowel Parasites* (*Oesophagostomum venulosum* and *Chabertia ovina*).—Only in the sheep treated with phenothiazine was there any evidence of efficiency against these parasites. In this group there was a reduction of 70 per cent. or more in the number of eggs of these parasites per gram of faeces in 12/22 or 54.5 per cent. of sheep. In 7/22 or 31.8 per cent. the reduction in egg count was less than 50 per cent.

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Studies on Phenothiazine as an Anthelmintic.

A Comparison of the Efficiency of Finely and Coarsely Ground Phenothiazine against *Oesophagostomum columbianum*.

*By Hugh McL. Gordon, B.V.Sc.**

Summary.

A coarsely ground preparation of phenothiazine appeared to be less efficient than a finely ground preparation against *Oe. columbianum* in sheep.

Introduction.

It was thought that there might be differences in the anthelmintic efficiency of phenothiazine related to particle size. On the one hand it seemed that a very finely divided powder might be dispersed more effectively among the contents of the alimentary tract, and thus be

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brought into more intimate contact with *Oe. columbianum*, while on the other hand coarser particles might be less affected by oxidation or other chemical change and so be more likely to reach the colon—the environment of the parasite—in higher concentration.

Siegler and Goodhue (1939)*, in studies on the effect of particle size of certain insecticides on their toxicity to codling moth larvae, found that coarse particles of phenothiazine were much less toxic than fine or medium particles (sizes of particles not given).

The Experiment.

Two groups of five young sheep, about 10 to 12 months old, were treated with a commercial phenothiazine known as Thiox, which contains about 93 per cent. of phenothiazine. The drug was administered in gelatine capsules, the dose rate being 0.6 g. per kg. body weight. Sheep in Group A received Thiox in its normal form, i.e., a very fine, barely palpable, powder. Sheep in Group B received Thiox which had been fused into large lumps and then ground until the particles passed through a sieve of 20 meshes to the inch but not through one of 30 meshes to the inch.

The sheep had been artificially infected with *Oe. columbianum* some months before the experiment.

Worms passed during the week following treatment were collected by attaching faeces bags to the sheep. The experimental sheep were then killed and the remaining *Oe. columbianum* were counted. The results are shown in Table 1.

TABLE 1.—SHOWING NUMBER OF *Oe. columbianum* PASSED IN THE FAECES AND NUMBERS DISCOVERED AT POST-MORTEM.

—	Sheep.	Worms Passed.	Worms Remaining.	Total.	Per cent. Removed.
Group A (Fine powder)	1	29	33	62	46·7
	2	72	9	81	88·8
	3	42	52	94	44·6
	4	26	..	26	100·0
	5	141	20	161	87·5
Group B (Coarse particles)	6	13	11	24	54·1
	7	1	9	10	10·0
	8	134	145	279	48·0
	9	118	53	171	69·0
	10	21	205	226	9·2

Quite large amounts of the coarsely ground Thiox were noticed in the faeces while washing to detect *Oe. columbianum*. It is possible, of course, that similar amounts of the finely powdered Thiox were passed but escaped notice. If the criterion of satisfactory efficiency is taken as 60 per cent. or more, treatment in Group A was satisfactory in three out of five cases (60 per cent.), while in Group B treatment was satisfactory in only one out of five cases (20 per cent.).

* Siegler, E. H., and Goodhue, L. D. (1939).—*J. Econ. Ent.* 32, pp. 199-203.

The Anthelmintic Efficiency of Lentin (Merck) against *Oesophagostomum columbianum*.

*By Hugh McL. Gordon, B.V.Sc.**

(From the Parasitology Laboratory, C.S.I.R., situated at the New England University College, Armidale, N.S.W.)

Summary.

Lentin (Merck) in doses exceeding those recommended by the manufacturers against "strongylus infestation" in sheep, was not an efficient anthelmintic against *Oesophagostomum columbianum*, *Haemonchus contortus*, or *Trichuris ovis*.

The use of this drug was attended with fatal toxic effects, six of the 22 sheep died, and a seventh recovered after administration of atropine.

These results, obtained in the field, confirm those of other trials carried out under laboratory conditions with sheep in pens and receiving "dry" feed (Edgar, loc. cit.).

Introduction.

Lentin (Merck), a choline derivative (amino-formyl-β-hydroxy-ethyl-trimethyl-ammonium chloride) was recommended by Oppermann (1937) for use against "strongyles" in sheep. Edgar (1939) has shown that, in doses slightly exceeding those recommended by the manufacturers, Lentin was not an efficient anthelmintic and, further, that in poor conditioned sheep it was highly toxic. His experiments were restricted to sheep maintained in pens and fed a ration of "dry" feed (chaff, oats, hay). The experiment described below was designed to supplement those of Edgar (loc. cit.) by using sheep running on natural pastures.

The chief pharmacological effect of Lentin is purgation. It has been shown (Gordon, 1939) that purgation alone is not an efficient method of removing *Oe. columbianum* from sheep. Lentin also promotes an outpouring of mucus from the mucous membrane of the intestinal tract, and it appeared that this effect, together with purgation, might dislodge large bowel parasites.

The Experiment.

The sheep used were running on a fresh growth of natural pasture during the fortnight preceding and during the trial. Sheep numbers 1 to 8, inclusive, were aged ewes in fair condition; numbers 9 to 14, inclusive, were aged wethers in poor to fair condition, while numbers 15 to 22, inclusive, were five months old and in fair to good condition. The sheep were starved from 10 a.m. on 16th April, 1939, and received the first treatment at 10.15 a.m. on 17th April, 1939. They were kept away from feed and water until a second treatment had been administered at 3.20 p.m. on 17th April, 1939. Bags were attached and all faeces passed during the next five days were collected and examined for the presence of *Oe. columbianum* and other nematodes.

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Results of Treatment against *Oe. columbianum.*

TABLE 1.—SHOWING DOSE RATES, NUMBERS OF *Oe. columbianum* PASSED, DISCOVERED AT POST-MORTEM, TOTAL AND PERCENTAGE REMOVED BY TREATMENT.

Dose Rate.	Sheep No.	Worms Passed.	Worms at Post-mortem.	Total.	Per cent Removed.
Group A (1)—1 ml. 0·1 per cent. solution repeated	4	2	145	147	1·3
	13	2	22	24	8·3
	1	..	73	73	0·0
	11*	17	34	51	33·3
	3*	8	536	544	1·5
Group A (2)—1 ml. 0·1 per cent. solution first dose, 1·5 ml. second dose	9*	9	223	232	3·8
	10	41	200	241	17·0
	2	5	161	166	3·0
	12	..	65	65	0·0
	5	5	97	102	4·9
Group B (1)—4 tablets repeated ..	6	7	35	42	16·6
	14	..	109	109	0·0
Group B (2)—4 tablets first dose, 6 tablets second dose	7*	7	97	104	6·7
	8*	61	31	92	66·3
Group C (1)—0·5 ml. 0·1 per cent. solution repeated	17	2	16	18	11·1
	15	14	18	32	43·7
	22	..	8	8	0·0
Group C (2)—0·5 ml. 0·1 per cent. solution first dose, 1 ml. second dose	18	37	17	54	68·5
	16	2	6	8	25·0
	19	4	14	18	22·2
Group D (1)—2 tablets first dose, 3 tablets second dose	21*	..	18	18	0·0
Group D (2)—2 tablets first dose, 4 tablets second dose	20	8	1	9	88·8

* Sheep died.

Tablets were given by mouth and solutions by subcutaneous injection.

N.B. :—Doses recommended by Merck for sheep with "strongylus infestation":

Sheep.—0·5 ml. 0·1 per cent. solution or 2 tablets, repeated on same day.

Lambs.—1 tablet repeated on same day.

All sheep showed marked salivation beginning within 10–15 minutes after administration of the drug. In those receiving tablets the onset of salivation was delayed, but did not exceed 30 minutes. Faeces became soft to diarrhoeic within half to one hour after administration of the drug.

It was necessary to administer atropine (the antidote for Lentin poisoning—1/10 gr.) to number 1 at 5 p.m. on 17th April, 1939, and number 8 at 10.30 a.m. on 18th April, 1939. Number 8 was then in a prostrated condition and died a few hours later. Numbers 11, 3, 9, 21, and 7 also died. The survivors were killed on 22nd April, 1939, and all remaining worms were counted.

Summary.

1. *Mortalities.*—Six out of 22 sheep died (27.2 per cent.). Two died within a few hours of the second treatment, three during the night following the second treatment, and one during the next night. It was necessary to treat two sheep with atropine; one died, and one recovered.

Sheep which died showed inflammatory changes in the colon and sometimes in the abomasum, and these organs were usually greatly distended with gas or ingesta.

2. *Efficiency against Oe. columbianum.*—Treatment was considered to be efficient if it removed 60 per cent. or more of these worms.

Adult Sheep.

Group A.—0.1 per cent. Lentin subcutaneously.

(1) 1 ml. repeated (five sheep). In no case was treatment efficient. Two sheep died.

(2) 1 ml. first dose, 1.5 ml. second dose (five sheep). In no case was treatment efficient. One sheep died.

Group B.—Lentin tablets by mouth (1 tablet = 5 mgm. Lentin).

(1) Four tablets repeated (two sheep). In neither sheep was treatment efficient. No deaths.

(2) Four tablets first dose, six tablets second dose (two sheep). Both sheep died, one after passing 66.3 per cent. of the *Oe. columbianum* harboured.

Young Sheep.

Group C.—0.1 per cent. Lentin subcutaneously.

(1) 0.5 ml. repeated (three sheep). In no case was treatment efficient. No deaths.

(2) 0.5 ml. first dose, 1 ml. second dose (three sheep). Treatment was efficient in one sheep out of three. No deaths.

Group D.—Lentin tablets by mouth.

(1) Two tablets first dose, three tablets second dose (one sheep). Sheep died, no worms removed.

(2) Two tablets first dose, four tablets second dose (one sheep). Treatment was efficient and the sheep survived.

It is thus seen that treatment was efficient, i.e., removed 60 per cent. or more of the *Oe. columbianum* present, in three out of 22 sheep (one of these three sheep died). The distribution of deaths among the groups receiving different dose rates appears to indicate that the higher dose rates were excessive, while it was only with these high and extremely dangerous doses that any appreciable anthelmintic efficiency was obtained. From these results, it appears that Lentin is not an efficient anthelmintic against *Oe. columbianum,* and that its use is attended with fairly considerable risk of causing mortality in sheep.

3. *Efficiency against Haemonchus contortus.*—This was controlled by faecal egg counts, by larval cultures made before and after treatment, and by observation of the worms of this species remaining at post-mortem examination. There was no indication that treatment with Lentin had removed more than a few *H. contortus.*

4. *Efficiency against Trichuris ovis.*—Treatment removed 100 per cent., 78.5 per cent., and 3 per cent. respectively of these worms from three sheep, and none from 10 other sheep (number of *T. ovis* present in these sheep ranged from 1 to 58).

5. *Efficiency against Chabertia ovina.*—A few sheep carried very light infestations with this species. One sheep passed two out of the three *C. ovina* which it harboured, while in three other sheep, harbouring 1, 1, and 17 *C. ovina* respectively, none of these worms were removed. These infestations were so light that it is difficult to draw definite conclusions regarding efficiency against this species.

Acknowledgments.

It is a pleasure to acknowledge the co-operation of those graziers who generously donated sheep for this experiment and the assistance in carrying out the trial which was rendered by Messrs. I. W. Montgomery and L. K. Whitten (Division of Animal Health and Nutrition, C.S.I.R.), and C. Blumer (District Veterinary Officer, (Armidale) and C. J. O'Neill (Inspector of Stock, Armidale), both officers of the Department of Agriculture, N.S.W.

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Scientific Papers from the Division of Economic Entomology published elsewhere than in the Council's Publications.

In previous issues (5: 184, 1932, and 7: 215, 1934) articles appeared discussing scientific papers for which officers of the Council's Division of Economic Entomology were responsible. The article that follows brings the former list of such publications up to date. It will be seen that the various papers are concerned, in general, with scientific aspects of the problems, such as that of the blowfly and the grasshopper, which the Division is investigating.—Ed.

Currie, G. A., 1937.—Galls on Eucalypt Trees. A new Type of Association between Flies and Nematodes. *Proc. Linn. Soc. N.S.W.*, **62**: 147–174.

Many galls on leaves and stem tips, leaf buds and flower buds of Eucalyptus trees are caused by the combined action of nematode worms and small flies of the Agromyzid genus *Fergusonina*. The flies and nematodes are invariably found together in the gall, and their relationship is described as a true symbiosis. The life histories of the flies and the nematodes have been worked out and their interdependence

revealed. The fly larvae, which are described for the first time, carry chitinous structures on the dorsum, and these are of great taxonomic value and phylogenetic interest. The nematodes are found in the galls as free-living females and males. The first generation living in the galls is composed of parthenogenetic females, and there is an alternation of generations during which a generation of fertilized females is parasitic in the adult fly. The adult fly deposits the nematode larvae in buds with her own eggs. The taxonomics and affinities of the nematodes are discussed and a new sub-genus *Anguillulina* (*Fergusobia*) is erected to contain them. Insect parasites of the flies are common and their significance in controlling the number of flies is discussed.

Fuller, M. E., 1937.—Notes on the Biology of *Tabanus froggatti*, *T. gentilis*, and *T. neobasalis* (Diptera). *Proc. Linn. Soc. N.S.W.*, **62**: 217–229.

Figures and detailed descriptions of the previously unknown larvae and pupae of these three blood-sucking flies are given, together with observations on their life histories and habits. The larvae are terrestrial whereas most other known *Tabanus* larvae are aquatic or semi-aquatic, and the larvae differ in two major morphological characters from the recorded European and American species: (1) the thoracic segments are unstriated on the dorsum, and (2) the posterior end is truncated.

Fuller, M. E., 1938.—On the Biology and Early Stages of *Helicobia australis* (Sarcophaginae), a Dipterous Insect associated with Grasshoppers. *Proc. Linn. Soc. N.S.W.*, **63**: 133–138.

Helicobia australis was recorded as a parasite of grasshoppers, but the observations recorded in this paper show that this species develops normally in dead grasshoppers and probably also in other dead insects. There is no evidence that it can parasitize living grasshoppers. Large numbers of the fly were bred in captivity. The larval instars and the puparium are described and compared with *Sarcophaga*. Interesting observations on the biology of the fly are recorded.

Fuller, M. E., 1938.—Notes on *Trichopsidea oestracea* (Nemestrinidae) and *Cyrtomorpha flaviscutellaris* (Bombyliidae)—two Dipterous Enemies of Grasshoppers. *Proc. Linn. Soc. N.S.W.*, **63**: 95–104.

The larvae of these two flies are the first of the Australian Nemestrinidae and Bombyliidae to be described. The first, *Trichopsidea oestracea*, was recorded as a parasite of the wingless grasshopper *Perelytrana rana*, and experiments at Canberra have shown that it parasitizes a number of other species including the Australian Plague Locust. The second, *Cyrtomorpha flaviscutellaris*, is recorded as a grasshopper parasite for the first time from *Austroicetes cruciata* in Western Australia. Figures and descriptions of the characteristic features and photographs of the larvae and pupae of both species are given.

Fuller, M. E., and Lee, D. J., 1938.—A new termitophilous Phorid (Diptera). *Proc. Linn. Soc. N.S.W.*, **63**: 75–80.

The termitophilous habit is fairly common in this group of flies, two of the Australian species are known to be associated with termites, and a new species *Diploneura gynaptera* is described and figured in this paper. This species was bred from larvae collected in the mounds of *Eutermes exitiosus*.

Gay, *F. J.*, 1938.—A nutritional Study of the Larva of *Dermestes vulpinus* F. *J. exp. Zool.*, **79**: 93–107.

This paper gives an account of experiments on the basic food requirements of the scavenger bettle *Dermestes vulpinus*. The beetle commonly attacks stored hides and skins, hard dry skins of carcasses, meat, cheese, and other similar materials of high protein content. It is shown that it can be reared from the egg to the adult stage on a diet of casein, cystine, cholesterol, yeast, and salts, or yeast, cholesterol, and salts. Cholesterol is essential for normal growth, vitamin B (undifferentiated complex) also appears to be necessary, and the salts contained in brewer's yeast are satisfactory for growth. *D. vulpinus* can develop on low concentrations of cystine.

Hill, G. F., 1938.—Sir Joseph Banks Islands. 9. Isoptera. *Proc. Roy. Soc. Vic.*, **50** (2): 353–355.

This short paper contains an account of the termites collected by the McCoy Society's expedition to the Sir Joseph Banks Islands in December, 1936. Four species are recorded, *Calotermes* (*Calotermes*) *condonensis*, *Heterotermes ferox*, *Eutermes exitiosus*, and *Hamitermes neogermanus*, and notes on the biology and distribution of each species are given.

Lee, D. J., 1937.—Notes on Australian Mosquitoes (Diptera, Culicidae). Part IV. The Genus *Theobaldia*, with Description of a new Species. *Proc. Linn. Soc. N.S.W.*, **62**: 294–298.

Five members of this genus have been described from Australasia and a sixth and new species, *Theobaldia inconspicua*, is described in this paper. Notes on the previously known species are given, and a key to distinguish the females of the Australasian species is included. Diagnostic features are illustrated by nine text-figures.

Mackerras, I. M., 1937.—Notes on Australian Mosquitoes (Diptera, Culicidae). Part III. The Genus *Aedomyia* Theobald. *Proc. Linn. Soc. N.S.W.*, **62**: 259–262.

This genus includes a small number of rare, ornate species, well separated from other genera and widely distributed. Two species which occur in Australia, *A. catasticta* and *A. venustipes*, are described in this paper.

Norris, K. R., 1938.—West Australian Mydaidae (Diptera). *J. Roy. Soc. W.A.*, **24**: 43–49.

This paper contains notes on five species of mydaid flies and in addition a new species, *Miltinus mackerrasi*, is described and figured.

RATCLIFFE, F. N., and CUMMINS, J. E., 1939.—Termite (White Ant) Research in Australia. *Empire For. J.*, **18**: 221–228.

This article is in the nature of a general review of the main lines of work at present being carried out, chiefly at Canberra, on termite problems. The more important economic species are named, and their habits briefly discussed. The problem of the prevention or reduction of termite damage to timber in service is considered under the following heads:—(1) constructional methods, (2) the use of chemical wood preservatives, (3) the use of timbers possessing a natural resistance to termite attack, and (4) direct control by poisons. The methods of testing the resistance of samples in the field and in the laboratory are briefly described, and the complications arising from the difference in habits of the different species of termites are referred to. Insofar as the results of specific investigations are mentioned, they represent the state of affairs in early 1939, since when considerable progress has been made in certain lines of work.

TONNOIR, A. L., 1937.—Revision of the Genus *Fergusonina* Mall. (Diptera, Agromyzidae). *Proc. Linn. Soc. N.S.W.*, **62**: 126-146.

Although this genus of gall-forming flies was erected in 1924, a revision has already become necessary on account of the many species revealed by the work of Currie on *Eucalyptus* galls (see above). In this paper the author gives a key to the nineteen species now known and a detailed description of each species, including eleven new species. There is no doubt that a large number of other species are likely to be discovered as each species of *Eucalyptus* seems to have its corresponding species of *Fergusonina*, and in some species several of them, according to the part of the tree from which the galls were found.

TONNOIR, A. L., 1938.—On the Taxonomy of *Helicobia australis* (Sarcophaginae), a Dipterous Insect associated with Grasshoppers. *Proc. Linn. Soc. N.S.W.*, **63**: 129–132.

The taxonomy of this fly, bred from dead grasshoppers (see Fuller above), is discussed, and it is considered that it can reasonably be placed in the genus *Helicobia*. The female of the species *H. australis* is described for the first time and the author also gives notes on the morphology of the male.

TONNOIR, A. L., 1939.—Psychodidae. British Museum (Natural History), Ruwenzori Expedition 1934-5, **1** (4): 35–80.

This paper describes the Psychodidae (moth and sand flies) collected by the Ruwenzori Expedition to equatorial Africa. Sixty species are recorded of which twenty-two are described for the first time, and 156 text-figures are included to illustrate the diagnostic features. In all, sixty-one species of *Phlebotomus* are now known to occur in tropical Africa. The main feature of the list is the absence of *Pericoma* and *Trichomyia*, which are genera with a wide distribution in other regions of the world. The most notable addition is that of *Bruchomyia*, so far known only from the neotropical region; its validity is discussed.

Waterhouse, D. F., 1939.—Temperature Preference in the Australian Sheep Blowfly, *Lucilia cuprina* Wied. *Aust. J. Sci.,* **11**: 31–32.

During a series of laboratory studies on the sheep blowfly *Lucilia cuprina,* it became clear that this species has a definite preference for a certain limited range of temperature. Thermometers were hung against the muslin wall of a cage through which the heating apparatus, a radiator, was directed, thereby giving a temperature gradient from the area directly opposite the radiator down to the normal room temperature; the preferred zone of temperature was found to be from 31.5° to 38°C. These observations were made in fairly dim, artificial light. When the room was flooded with bright daylight, the flies were stimulated to intense flight activity, and, unless the temperature was below about 15°C., they did not settle long enough anywhere for zoning to be apparent.

Wilson, F., 1938.—Some Experiments on the Influence of Environment upon the Forms of *Aphis chloris* Koch. (Aphididae). *Trans. R. ent. Soc. Lond.* **87**: 165-180.

While investigating the insect enemies of St. John's Wort, *Hypericum perforatum,* in England, *Aphis chloris* was discovered. Experiments were undertaken to determine the factors which induce and inhibit the appearance of different forms of the insect during the course of its annual cycle. There are five forms of this aphid—the fundatrix, the apterous viviparous female, the alate viviparous female, the male, and the oviparous female. Alate viviparous females are not essential to the cycle. Alatae occur as a result of particular environmental conditions, e.g., overcrowding, which is interpreted as being a nutritional factor. Males seldom appeared during the experiments, and it is suggested that the drastic conditions under which the lines were maintained favoured the viviparous females, or, at the other extreme, favoured the oviparous females. Evidence from the field is given to support the view that the environmental conditions favourable to the males lie between those favouring the viviparous females and those favouring the oviparous females.

Wilson, F., 1938.—Notes on the Insect Enemies of Chermes, with particular reference to *Pineus pini* Koch. and *P. strobi* Hartig. *Bull. ent. Res.,* **29**: 373–389.

Pine Chermes was probably introduced into Australia with its coniferous host from the northern hemisphere; consequently a study of the natural enemies of Chermes was made in England. The results of this work are reported in this paper. No parasites were found but numerous predators exist, the most important of which appear to be—(1) *Leucopis obscura* Hal. (Ochthiphilidae), (2) *Lestodiplosis pini* Barnes (*Cecidomyiidae*), (3) *Hemerobius stigma* Steph. (Hemerobiidae), (4) *Wesmaelius concinnus* Steph. (Hemerobiidae), (5) *Exochomus quadripustulatus* L. (Coccinellidae). Details of the life histories and habits of these insects are given in this paper together with biological notes on a number of other minor predators.

Experiments on the Recovery of Sheep Nematode Larvae from Pastures.

*By G. P. Kauzal, D.V.Sc.**

A satisfactory method for recovering from pastures the larvae of worms which parasitize sheep and other domestic animals has long been needed. Worms do not multiply within their hosts, as do bacteria, and every worm found within an animal has been ingested, generally as a larva, with the food or water. Hence curative treatment by drugs is not enough, and the animal so treated will quickly become reinfested if kept on contaminated pastures. Until such techniques as that which is described in this paper have been perfected, it is impossible to gauge the time required by worm-infected pastures and soils to become free, or relatively free, of worm larvae. Thus such work is of great importance in enabling a proper understanding of the longevity of the various parasitic worms during that stage of the life-cycle which is passed in the outer world. Only with such an understanding can adequate and reasoned control measures, such as the spelling of pastures and rotational grazing, be soundly planned.—Ed.

Summary.

A short *résumé* of the literature is given in order to see if any technique used by others could be successfully adopted in isolating larvae and eggs of sheep nematodes from soil and pastures. Unfortunately, no satisfactory methods were revealed, and this necessitated a new series of experiments being made.

Among the experiments described, there is one technique which gave very much more satisfactory results than others. The apparatus is shown in Fig. 2 and is also described.

In principle, the isolation relies upon the fact that larvae migrate laterally as well as in a vertical direction from the soil.

Introduction.

The importance of recovering infective larvae of nematode parasites of sheep from grazing land is twofold, namely, to facilitate studies of the longevity of eggs and larvae under various conditions, and to enable a "diagnosis" of the degree of infestation with eggs and larvae of particular areas of grazing land. Apart from establishing the degree of infestation with reasonable accuracy, the technique must be such that it will be possible to determine the species of infective larvae which are present. Only with an adequate knowledge of the longevity of nematode eggs and larvae can measures (such as rotational grazing) be used to full advantage in association with specific anthelmintic treatment of the sheep.

Infective material can accumulate in certain areas according to the nature of the country. This was evident from a preliminary survey made by the author in 1934 on a 15-acre paddock which was

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stocked at a rate of one sheep to the acre. Samples were collected periodically from the vicinity of pegs inserted in areas representing average grazing land (generally level), hill top, hillside, creek-side, and creek-bed. Attention was also given to the nature of the grass cover of the areas sampled (short, long, bare, &c.). Infective larvae of species of *Haemonchus, Trichostrongylus, Chabertia, Oesophagostomum,* and *Nematodirus* were recovered in varying numbers from low-lying areas adjoining hills, or from areas showing accumulation of pellets, obviously deposited by storm water after rain. The hillside and hilltop showed smaller larval populations, except where the sheep regularly camped each night.

The results of this survey clearly showed that such observations would be valuable provided a reliable technique for the recovery of larvae could be devised, and it was therefore decided to concentrate on perfecting such a technique as a necessary prelude to further field investigations.

There is also an urgent need for an efficient technique whereby the *potential* infectivity of a grazing area can be predetermined by demonstrating the number of viable eggs which may develop up to the infective larval stage under favourable weather conditions.

The technique, therefore, may be designed according to the aspect one wishes to investigate, namely:—

(*a*) The number of already developed infective larvae which threaten the sheep at the time the sample is collected.

(*b*) The number of eggs which, under favourable weather conditions, would add to the already existing infective material.

(*c*) The number of eggs and larvae combined.

Having these three main aspects in mind, investigation was made into the possibility that one or other of the techniques which have been described by other workers might fulfil our requirements. Some of them were modified in an attempt to improve their efficiency, and certain new methods were also devised and tested. It must be remembered, however, that the larvae which were used in the experiments of Baermann, and several others whose work is referred to herein, are skin penetrators, and hence may respond to a given technique better than most of the sheep nematode larvae, which, with the exception of *Gaigeria, Bunostomum,* and *Strongyloides,* are not skin penetrators.

Before describing the author's own experiments, the work which has been done by others in this direction must be briefly reviewed.

Baermann (1917) described a method by which larvae of the human hookworm could be isolated from polluted areas or from human faeces. His original description was not available to the author, but a re-description of the technique was published by Cort, Ackert, Augustine, and Payne (1922). According to these authors, Baermann used a small funnel containing a sieve 7.5 cm. in diameter and 3.5 cm. in depth. By filling the funnel with water so

that the floor of the sieve was immersed, attempts were made to isolate larvae from small amounts of soil (50–100 gm.) lying on the sieve. The apparatus is shown schematically in Fig. 1.

BAERMANN'S APPARATUS FOR RECOVERY OF NEMATODE LARVAE FROM SOIL PLOTS.

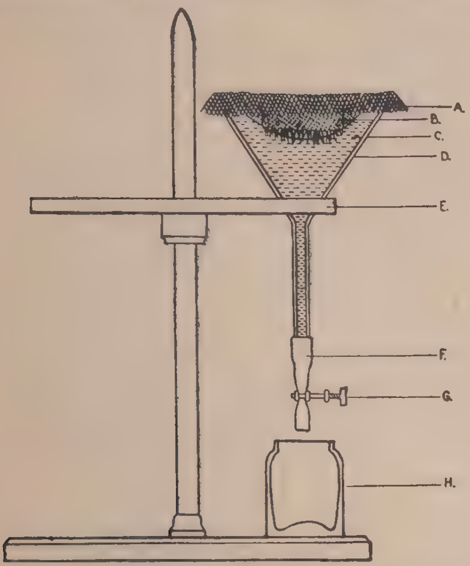

FIG. 1—(a) wire gauze; (b) soil plot (grass facing downward); (c) water in which the grass is immersed; (d) funnel; (e) stand; (f) rubber tubing; (g) clamp; (h) glass jar.

The results of these experiments were apparently satisfactory. In one of them seven larvae were recovered out of eight placed on the soil, but it was found that, in general, the results with this technique were most erratic. Efficiency was increased by using warm water, and by mixing coarse gravel and clay-loam with the soil from which the larvae were to be isolated. In one case this latter method yielded 100 per cent., while in two other cases the percentage recoveries were 93 and 81 respectively. Indeed, the results shown here appear good, but the percentage of recovery in the rest of their experiments varied from 4 per cent. to 100 per cent.

A more elaborate modification of Baermann's technique was used by Thiel and Wolff (1931). It consisted of a cylinder of wire gauze closed at one end and lined with paper soaked in 0.5 to 0.75 per cent. agar. On the agar floor was placed a layer of sand 3 mm. deep which was kept moist by standing the cylinder in a Petri dish containing a small amount of water. The whole apparatus was kept at 55°–60°C. It was expected that hookworm larvae would penetrate through the agar layer into the water in the Petri dish, whereas the larvae of the soil nematodes would remain behind on the agar floor. It is claimed that the technique was successful in this regard.

Spindler (1929), in order to make a quantitative estimation of the number of *Ascaris* eggs present in soil, used sodium dichromate to float the eggs on to the surface after centrifuging the mixture for one to two minutes.

In studying the migration of the infective larvae of *Nippostrongylus muris* of the rat, Africa (1931) used a dish which was half filled with sterile garden soil. A circular piece of filter paper, about an inch in diameter, was moistened and placed in the centre of the soil. Freshly-passed eggs were placed on the filter paper, after which the whole apparatus was covered and set aside at room

temperature. After the seventh day numerous larvae appeared at the edge of the soil near the sides of the dish. With this technique, Africa was able to demonstrate the lateral migration of the infective larvae of *N. muris*. The possibility of adapting this technique for quantitative estimation of infective nematode larvae of sheep present in soil was considered by the author, and the results of these experiments will be discussed below.

Ross and Kauzal (1932), while working on the life-cycle of *Stephanurus dentatus*, found that the original Baermann isolation method, when modified, was effective in separating the infective larvae of this species from the soil.

Likewise, Parnell (1936), while working on the effect of various chemicals on the infective larvae of certain nematodes affecting horses and sheep, has adopted the Baermann technique as a means of ascertaining the number of surviving larvae. The method was applied at intervals to faeces which had been treated with the various chemicals, until the number of larvae recovered was considered negligible. It was found that the technique gave very inconsistent results, and that when attempts were made to recover larvae from faecal samples to which known numbers had been added, the percentage recovered depended greatly on the number of larvae which had been added. Thus when 75–100 larvae were used, the percentage recovery ranged from 12.5 per cent. to 45 per cent.; when 1,000 were used from 35 per cent. to 50 per cent. were recovered, and when 10,000 were used the percentage recovered varied from 45 to 90. This was the first attempt to determine the efficiency of the Baermann technique as applied to infective nematode larvae of the domestic animals.

Several methods have been tried to separate insect fauna from the soil, and Ladell (1936) has described one method which is apparently very effective. In principle it is a flotation method using a 25 per cent. magnesium sulphate solution. The whole process is carried out in a rather elaborate apparatus, whereby the floating insects are eventually collected on a filter paper in a Buchner funnel.

It is obvious from the brief review just given that a reliable and efficient technique for ascertaining the number of living and infective larvae present on a unit of grazing land was yet to be devised.

The Author's Experiments.

It is not necessary to discuss details of the numerous preliminary experiments which have been carried out since the commencement of this work in 1934, mainly because the results obtained were not satisfactory. Statistical analysis of results up to 15th June, 1936, showed that one could usually recover about 35 per cent. of the larvae used to infect the experimental plots. The larvae used during these investigations were chiefly *Haemonchus contortus*, but mixtures of *Haemonchus* and *Trichostrongylus* spp. were also used.

The technique most frequently tried was, in principle, that originally described by Baermann; several alterations were made, however. The soil plots used throughout have been of 71 mm. diameter, representing one-millionth of an acre. It was found that water at room temperature (15° C.–22° C.) was more effective than water heated to body temperature (37° C.). The size of the funnel, compared with the amount of soil used, received attention, as it was thought that more larvae could be isolated from a larger funnel having a larger area available on which to spread the soil. In this latter regard results may be summarized as follows:—Using a 6-in. funnel and 50 gm. of soil the average recovery from eleven experiments was 33.6 per cent. of larvae exposed, whereas the same size of funnel yielded an average of only 17 per cent. of larvae in the same number of experiments when 100 gm. of soil was used. Using a 9-in. funnel and 100 gm. of soil in a further seven experiments, the recovery increased to 43 per cent. All these

tests were carried out under identical conditions, using water at room temperature and an isolation period of 48 hours.

Other experiments were designed to determine whether gauze used to support the soil on top of the funnel retained any of the larvae. They showed that the mean recovery when the soil was supported with gauze was 36.7 per cent., whereas when no gauze was used it was 58.3 per cent.

It is evident from the foregoing that, although valuable information was obtained from these experiments, the percentage recovery as a whole was low. In addition, the percentage recovered in individual experiments varied greatly.

In an attempt to improve the recovery rate and to secure more uniform results, various methods were tested, their general purpose being (*a*) to attract the larvae from the soil and grass blades, or (*b*) to remove them mechanically.

Attempts were made to attract the larvae towards the lower end of the funnel of the Baermann apparatus by blackening all but the lower portion of the funnel's stem and covering the top with black paper. It was hoped that the larvae would migrate towards the light entering through the unblackened portion. This proved unsuccessful, however, and no better result was obtained when heat as well as light was supplied at the neck of the funnel by means of an electric bulb.

Solutions having a high specific gravity, namely, glycerine, saturated solutions of sugar, salt, and sodium nitrate were tested in an endeavour to float the eggs and larvae to the surface. Although this method is commonly used for diagnostic examination on faecal samples, it was found quite unsatisfactory for our purpose. Firstly, so much debris was carried to the surface that it was difficult to count the larvae and eggs that were mixed with it. Secondly, the solutions adversely affected the vitality of larvae so that differentiation between living and dead ones was impossible. Moreover, the percentage recovery when known numbers of larvae were exposed was very low; for example, in one experiment using saturated solution of sodium nitrate and 1,200–1,400 larvae, less than 7 per cent. were recovered.

An apparatus was constructed consisting of a coil of copper tubing perforated on one side and through which, by connecting it with a water tap, a fine spray could be played on the surface of the soil plot. This coil was inserted in the funnel so that it lay just below the grass-covered surface of the soil plot. A bent glass tube was used to connect the bottom of the funnel with a second funnel which was closed at its lower end with rubber tubing and clamp. The heavier debris collected in the first funnel while much of the lighter material passed on into the second funnel. When both funnels were full, washing was discontinued and, after standing, the sediment in both funnels was examined for larvae. The recovery rates from one set of plots varied between 64 and 67 per cent., while from another set it ranged from 25 to 60 per cent. Although these results were somewhat erratic, the rather high recovery rate suggests that further investigation should be made on these lines.

It was found that infective larvae, when suspended in water together with soil particles and left to sediment, were found in the water just above the heavier soil sediment. This is no doubt due partly to their being lighter and partly to their active movement. When the

supernatant water was poured off from such a preparation, the majority of the larvae were removed with it, leaving the sediment behind. It was hoped to devise a method for isolating larvae from soil plots based on this observation and using a special trough with a corrugated floor designed for sedimenting and washing the sediment. Although some progress has been made, it is as yet insufficient for conclusions to be drawn.

Description of Experiments which have Particular Significance.

Recovery of Larvae by the Double Petri Dish Bell-jar Technique.

1. Encouraging results have been obtained recently by placing the plot from which the larvae are to be recovered in a Petri dish, inserting this in a second, somewhat larger, Petri dish, and covering with a bell-jar to maintain constant humidity. The apparatus is shown schematically in Fig. 2.

Arrangement for the Double Petri Dish and Bell Jar Technique.

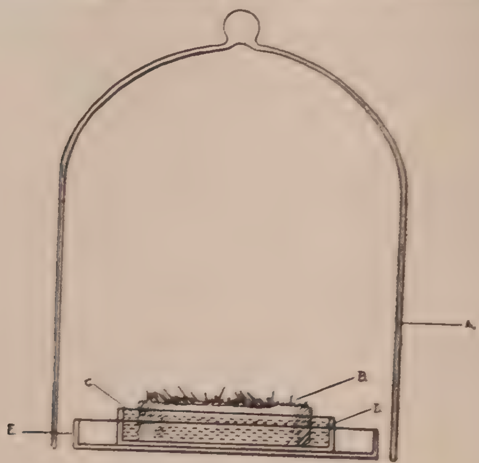

Fig. 2.—(a) bell jar; (b) soil plot inside inner petri dish; (c) water in which soil plot rests; (d) inner petri dish; (e) outer petri dish.

While this method was devised here as a result of independent observations, search of the literature reveals that Africa (1931) used a rather similar apparatus when studying the activity of the infective larvae of *Nippostrongylus muris*.

The inner Petri dish, which is about 1 inch smaller in diameter than the outer one, holds the grass plot, and into it is poured sufficient water thoroughly to saturate the plot and half fill the dish. The soil plot should be about 1 inch smaller in diameter than the dish which holds it, and as thick as the dish is deep. The "moat" of water in the dish serves to trap larvae leaving the surface of the plot, and the purpose of the outer dish is to catch any which climb over the sides of the inner dish.

In experiments with this apparatus, crumbled faeces containing an estimated number of eggs per gram were evenly scattered over the surface of the soil plots after trimming the grass thereon to an even level. After adding the requisite amount of water, the apparatus was covered with a bell-jar and set aside at room temperature (24°C. approximately) for seven days.

After this period, the grass was clipped close to the level of the soil and washed with water to remove any larvae that were upon it, the soil and grass roots were subjected to Baermann's isolation technique for four days, and the water in the Petri dish plus washings of the outer dish (including the outer surface of the inner dish) were also collected.

Controls were contrived by submitting similar quantities of the same supply of faeces to identical conditions except that the inner dish contained faeces only, which were kept slightly moist. The results obtained in the first experiment with this method may be tabulated as follows:—

Table 1.—The Recovery of Larvae from Soil and Grass Contaminated with Known Numbers of *H. contortus* Eggs in Crumbled Faeces.

Soil Plot Number.	Recovery made from—	Number of Larvae Recovered.	Percentage of Recovery.	Total Number Recovered.	Percentage of Larvae Recovered Compared with the Number of Eggs Originally Exposed.
1	Grass ..	367	7	5169	31
	Soil	1202	23		
	Water in dish	3600	70		
2	Grass ..	115	2	4408	27
	Soil	1031	20		
	Water in dish	3262	78		
3	Grass ..	47	0·7	6377	38
	Soil	2970	46		
	Water in dish	3360	53·3		
4	Grass ..	37	0·4	9027	54
	Soil	990	10		
	Water in dish	8000	89·6		
5	Grass ..	21	0·4	5147	31
	Soil	2726	53		
	Water in dish	2400	46·6		
6	Grass ..	15	0·2	5395	33
	Soil	480	9		
	Water in dish	4900	90·8		

TABLE 2.—RECOVERY OF LARVAE FROM CONTROL DISHES CONTAINING CRUMBLED FAECES WITH A KNOWN CONTENT OF *H. contortus* EGGS.

Control Number.	Recovery made from*—	Total Number of Larvae Recovered.	Percentage of Recovery.
1	Faeces placed in inner dish ..	5500	33
2	" " " ..	5940	36
3	" " " ..	6175	37
4	" " " ..	5250	32
5	" " " ..	7200	43
6	" " " ..	4725	28

* The number of larvae present was estimated by sampling of a thoroughly mixed suspension of the contents of the inner Petri dish and washings of both dishes.

The average number of larvae recovered from the six experimental plots was 5,925, whereas the average for the controls was 5,798. The recovered larvae, expressed as a percentage of the number of eggs exposed, was 36 for the six experimental plots and 35 for the six controls.

The variation between individual plots, as regards the percentage of eggs subsequently recovered as larvae, was from 27–58 in the experimental plots, and from 28–43 in the controls, and the range through which individual recoveries varied was fairly similar. The percentage of recovery in both the experimental plots and the controls may seem rather low (35–36 per cent.), but the relatively uniform results indicate strongly that by no means all the eggs which were exposed developed to the infective larval stage. The number of larvae recovered may well represent the major portion of eggs which did so develop.

It was thought that probably many of the eggs had not developed, but a search failed to detect any remaining in the faeces. Observations revealed, however, that eggs of *Haemonchus contortus*, freshly collected from sheep and kept under conditions which did not favour development, showed signs of degeneration from the fifth day onwards. Such signs were the gradual disappearance of the eggshell, and the cells taking on a dark, granular appearance or becoming vacuolated. Vacuoles seem to appear in the cells mainly when the eggs have undergone development to the stage where they contain fully developed embryos. Soon after degenerative changes appear, the eggs become unrecognizable. Failure to detect eggs in the faeces remaining after the process just described is, therefore, no indication that all have developed into larvae.

It thus appeared that a good recovery of larvae was obtained from the double Petri dish bell-jar technique when crumbled faeces containing an estimated number of *eggs per gram* were evenly scattered over the surface of the soil plots. It was, however, still doubtful if satisfactory recovery could be obtained by exposing *mature larvae* on the soil, and Experiments 2 and 3 were therefore set up to answer this question.

2. On this occasion, 1,145 infective larvae of *H. contortus* suspended in 2 ml. of water were used to infect each soil plot. The larvae were obtained from a seven-day-old faecal culture. Two ml. of the suspension was drawn into a pipette and transferred to the centre of the surface

of the soil plot, allowing the suspension to soak slowly into the soil at the base of the grass blades. It was found necessary to moisten the soil plot before the larval suspension was added; otherwise this suspension was likely to run off the plot. From this point onwards, the treatment of the soil plots was identical with that described for the first experiment, except that the time of exposure was reduced to four days.

An equal number of controls was set up with the same number of *H. contortus* larvae placed in the inner Petri dish without the presence of the soil plot.

To study the effect of varying temperature on the results obtained, half of the sets were maintained at room temperature (22°–26°C.), while the remaining half was placed into an incubator room in which the temperature ranged between 24°–28°C.

TABLE 3.—FINAL RATE OF RECOVERY OF LARVAE OF *H. contortus* WHEN LARVAE, AS SUCH, WERE USED TO INFECT SOIL PLOTS.

Exposed.	Soil Plot Number.	Recovery made from—	Number of Larvae Recovered.	Percentage of Recovery.	Total Number Recovered.	Average Number of Larvae Recovered.	Percentage of Larvae Recovered Compared with the Number of Larvae Originally Exposed.
Incubator room at 24° to 28°C.	1	Grass ..	30	4·1			
		Soil	82	11·3	728		63·6
		Water in dish	616	84·6		687	
	2	Grass ..	Neg.	..			
		Soil	100	15·5	646		56·4
		Water in dish	546	84·5			
Room temp.	1	Grass ..	72	8·8			
		Soil	304	37·0	821		71·7
		Water in dish	445	54·2		765	
	2	Grass ..	42	5·9			
		Soil	125	17·6	710		62·0
		Water in dish	543	76·5			

Average percentage total recovery = 63·4.

The average number of larvae recovered from the plots exposed at room temperature was slightly better than from those kept in the incubator room, being 765 and 687 respectively. On the whole, a satisfactory number of larvae were recovered, especially when one considers the number of motionless larvae derived from the controls. It can be assumed that there would also be many motionless larvae on the soil plots and, since it is the active movements of the larvae which are mainly responsible for their recovery by the technique in use, it follows that the majority of inactive ones would not be recovered.

TABLE 4.—RECOVERY FROM CONTROL DISHES IN WHICH KNOWN NUMBERS OF *H. contortus* LARVAE WERE EXPOSED IN WATERY SUSPENSION.

Exposed.	Dish Number.	Recovery made from—	Number of Active and Motionless Larvae.		Percentage of Active and Motionless Larvae.		Total Number Re-covered.	Percentage of Larvae Recovered Compared with Number of Larvae Exposed.
			Active.	Motion-less.	Active.	Motion-less.		
Incubator room at 24° to 28°C.	1	Suspension of larvae placed in inner dish	514	398	56·4	43·6	912	79·7
	2	Suspension of larvae placed in inner dish	528	415	56·0	44·0	943	82·4
Room temp.	1	Suspension of larvae placed in inner dish	542	480	53·0	47·0	1022	89·3
	2	Suspension of larvae placed in inner dish	592	368	61·7	38·3	960	83·3

Average percentage total recovery = 83·8.

Assuming that the same percentage of larvae were motionless on the experimental soil plots as were found in the control Petri dishes, the recovery from the soil plots, based on the number of active larvae, was apparently 100 per cent.

This conclusion is arrived at from the fact that the average percentage of motionless larvae in the control dishes was 43.2, whereas the average percentage of active larvae recovered from the experimental plots was 63.4. The total of these two (106.6 per cent.) should therefore theoretically represent the total number of larvae which were originally present on the soil plots, and which would have been recovered had the 43.2 per cent. remained active.

3. In this experiment, 870 infective larvae of *Trichostrongylus* spp. were exposed, suspended in 4 ml. of water. *Trichostrongylus* spp. were used in order to discover whether this technique will recover them from soil to a similar extent as the larvae of *H. contortus.*

Four soil plots and four control dishes were used, and the same procedure was followed as in Experiment 2, namely, half were placed in the incubator at room temperature (24°–28°C.) for four days while the others were set aside at room temperature for a similar period.

The results are tabulated as follows:—

TABLE 5.—FINAL RATE OF RECOVERY OF *Trichostrongylus* LARVAE WHEN USED, AS SUCH, TO INFECT SOIL PLOTS.

Exposed.	Soil Plot Number.	Recovery made from—	Number of Larvae Re-covered.	Per-centage of Re-covery.	Total Number Re-covered.	Average Number of Larvae Re-covered.	Percentage of Larvae Recovered Compared with the Number of Larvae Originally Exposed.
Incubator room at 24° to 28°C.	1	Grass ..	7	1·2	602	657	69·2
		Soil	25	4·2			
		Water in dish	570	94·6			
	2	Grass ..	12	1·7	713		82·0
		Soil	22	3·1			
		Water in dish	679	95·2			
Room temp.	1	Grass ..	13	2·0	645	669	74·1
		Soil	17	2·6			
		Water in dish	615	95·4			
	2	Grass ..	6	0·9	693		79·6
		Soil	22	3·2			
		Water in dish	665	95·9			

Average percentage total recovery = 76·2.

TABLE 6.—RECOVERY FROM CONTROL DISHES WHEN *Trichostrongylus* LARVAE ARE EXPOSED THEREIN IN WATERY SUSPENSION.

Exposed.	Dish Number.	Recovery made from—	Number of Active and Motionless Larvae.		Percentage of Active and Motionless Larvae.		Total Number Re-covered.	Percentage of Larvae Recovered Compared with Number of Larvae Exposed.
			Active.	Motion-less.	Active.	Motion-less.		
Incubator room at 24° to 28°C.	1	Suspension of larvae placed in inner dish	801	..	100	..	801	92·0
	2	Suspension of larvae placed in inner dish	644	..	100	..	644	74·0
Room temp.	1	Suspension of larvae placed in inner dish	675	..	100	..	675	77·6
	2	Suspension of larvae placed in inner dish	892	..	100	..	892	100·0

Average percentage total recovery = 85·9.

The difference between the average number of larvae recovered from the incubator room and those exposed at room temperature is insignificant, being 657 and 669, respectively. The average percentage of recovery for the experimental plots was 76.2, compared with 85.9 for the control.

There was a complete absence of motionless larvae in the control group, a fact which may be attributed to the more resistant character of *Trichostrongylus* spp. larvae. As there were no motionless larvae in the control lots, it can be assumed that the average recovery from the soil plots of 76.2 per cent. represents all that this technique could recover. The remaining 23.8 per cent. may be accounted for, at least in part, as inaccuracy in estimating the number of larvae exposed or to the distribution of larvae in the watery suspension being uneven. That the latter was a factor of considerable importance is indicated by the fact that the recoveries from the control dishes varied from 74 per cent. to slightly over 100 per cent. of the estimated number originally exposed.

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A Survey of the Problem of Hoary Cress (*Lepidium draba* L.) as a Weed in Victoria.

By E. J. Pemberton and R. W. Prunster, B.Sc. (Agric.)†*

1. Introduction.

During the latter part of last year, arrangements were made by the Victorian State Government for the Council for Scientific and Industrial Research to undertake investigations on the control of hoary cress (*Lepidium draba* L.), a perennial weed of arable land mainly in the 16 to 20-in. rainfall areas. Prior to this, a Weeds Co-ordination Committee had been formed in Victoria, comprising representatives of the Department of Crown Lands and Survey, the Department of Agriculture, and of the Council for Scientific and Industrial Research, and a local Weeds Committee had been formed at Murtoa and Rupanyup to assist and advise in the proposed hoary cress investigations.

These two bodies held their first meeting at Rupanyup on 17th August, 1939, and it was decided that a survey of the problem should be made as a first step in the new investigations. Information obtained from the Victorian Lands Department, discussion at this meeting, a search of Australian and overseas literature, and a knowledge of the farming areas affected, contribute to the survey. Since the bulk of the experimental work will be carried out in the Wimmera, more detail will be given in respect of this area.

2. Area Covered and Economic Importance.

Hoary cress has seriously reduced the value of high-priced wheat lands, is extremely difficult and costly to eradicate, very seriously reduces wheat yields in poor seasons, and is being rapidly spread by cultivation; it is at present considered to be Victoria's worst weed. It is of greatest economic importance in that portion of the central Wimmera bounded by Donald, Hopetoun, Natimuk, and Stawell, but it has been reported from almost every land district in Victoria.

Table 1 shows all land districts where areas of more than 300 acres infested with hoary cress are known to exist. Land inspectors have estimated the known area of cress-infested land in their respective districts and have arbitrarily classed the infestation as being "heavy" or "light." In some cases, notes have been supplied stating control measures on smaller areas. The total area of 93,881 acres represents one forty-fifth of the total cropped area in Victoria, and one twenty-sixth of the area annually sown to wheat.

It is held by Wimmera settlers that land valued at £12 to £17 per acre for wheat cultivation has depreciated with hoary cress infestation to £5 to £7 per acre, as grazing land unsuitable for wheat. These figures are probably extreme, and little consideration is given to the depreciation of normal wheat lands as a result of lower wheat prices

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in the past ten years. Data of the average effect of cress in reducing grain yields are difficult to obtain, but the fact that settlers with a higher proportion of grazing to cropped land are still able to obtain high grain yields despite heavy cress infestation, seems to indicate that the weed owes its economic importance to the difficulty of its eradication, rather than to the difficulty of its control or the loss of grain yield which need be due to it.

TABLE 1.—SUMMARY OF AREAS IN VICTORIA INFESTED WITH HOARY CRESS.

Land District.	Total Infestation.	Heavy Infestation.	Light Infestation.	Method of Treatment.
	Acres.	Acres.	Acres.	
Werribee ..	14,550	3,000	11,500	Irrigated pasture. Salt
Warracknabeal	22,400	4,620	17,780	Salt on small patches
Hopetoun ..	9,702	7,781	1,921	
Donald ..	1,730	1,730	..	Salt. Grazing. No cultivation
Beechworth ..	400	300	100	
Castlemaine ..	1,250	450	800	Cut at flowering
Stawell ..	2,904	1,620	1,284	Salt
Dunolly ..	5,260	2,865	2,395	
Euroa ..	320	105	215	Cultivation discontinued
Raywood ..	4,200	3,500	700	
Gisborne ..	600	..	600	
Geelong ..	1,050	300	750	
Maryborough	2,250	1,780	470	
St. Arnaud ..	1,370	1,180	190	
Wycheproof ..	486	..	486	
Numurkah ..	1,194	..	1,194	Cultivation discontinued
Colac ..	311	310	1	
Bendigo ..	695	450	245	Sown to pastures
Rainbow ..	1,290	700	590	
Bacchus Marsh	8,400	6,000	2,400	
Shepparton ..	910	450	460	
Nhill ..	318	206	112	Salt. Sown to grass
Horsham ..	7,700	..	7,700	
Sea Lake ..	352	..	352	
Durham Ox ..	1,715	1,320	395	
Ouyen ..	812	..	812	Salt
Miscellaneous	1,712	230	1,482	
	93,881	38,897	54,984	

3. Soils and Farming Practice of the Wimmera and their Relations to the Control and Spread of Hoary Cress.

Soils.—The soil types met with may be broadly classified into two, the grey (known locally as "black" soil) and the red. Of these, the grey soils cover by far the greater area. They are both soils of heavy texture and good depth with a considerable amount of lime in small nodular form at a depth of 9 inches.

The structure of the grey soils is such that wide, deep cracks (up to 4 inches wide and 4 feet deep) develop on drying. It is believed that this causes fracture of the horizontal underground stems of hoary cress, preventing deep penetration of chemicals and limiting the effects of cultivation.

The "Wimmera plains" were originally treeless, but the more undulating parts carried pine (*Callitris robusta* and *C. propinqua*), buloke (*Casuarina luehmani*), and belar (*Casuarina lepidophloia*), with typical box (*E. bicolor*) flats occurring.

Rotations.—The Wimmera is highly priced cultivation land, and the typical crop rotation is fallow, wheat, oats or barley (for hay or grazing), and stubble pasture. With lower wheat prices there has been a tendency to reduce the rotation period so that a fallow wheat system is common on many farms, and small sections of some farms have had as many as ten crops in twelve years.

Yields.—Wheat yields vary from 6 to 14 bags per acre in average seasons, and according to land, farming practice, and hoary cress infestation, though in poorer seasons yields drop to 1½ to 4½ bags per acre on badly infested holdings.

Fertilizers.—The usual application of superphosphate is 70 lb. per acre for wheat (approximately 14 lb. of available phosphate per acre). Since 0.75 lb. of phosphate is removed in each bushel of wheat, it is seen that all crops heavier than six bags per acre are removing soil phosphate, and a continual depletion is taking place. Sown pastures are rare, and top-dressing with superphosphate during the stubble pasture years is uncommon.

Grass paddocks have the appearance generally of being deficient in nitrogen, and the annual medics (*Medicago* spp.) are the only common legume. With the very favourable rains of 1939, some good swards were obtained (particularly where the paddocks have been top-dressed). Clovers are almost absent, and the medics are unreliable in less than average seasons.

Cultivation.—Cereals, for grain, are planted on well-prepared fallows, and the rigid-tyned scarifier used for spring and autumn cultivations is usually fitted with a knife bar. Harrows are used to improve the tilth.

There is no doubt that cultivation is the major factor in the spread of hoary cress. This is appreciated by farmers, and it is not uncommon to see as many as 100 oval-shaped, uncultivated, cress-infested patches in a fallowed field.

It also seems certain that a short rotation without grazing and an inadequate use of phosphatic fertilizers play an important part in permitting cress to compete successfully against wheat for nutrients and moisture in the early part of poorer seasons.

4. Previous Experimental Work and Present Means of Control.

Morgan, working at the State Research Farm, Werribee, carried out a series of experiments, the results of which have been published in the *Journal of the Department of Agriculture, Victoria*, between 1931 and 1934.

The results of these experiments may be briefly summarized, as follows:—

(i) *Salting.*—At Werribee salt applications of the order of 7½ tons per acre did not give a satisfactory kill of hoary cress. This is attributed to the heavy clay sub-soils preventing penetration of chloride to more than a few inches deep.

(ii) *Study of depth of regeneration.*—By excavating a trench varying in depth from 6 to 36 inches, and re-filling with clean soil, it was found that the plant would not regenerate from underground stems cut off at depths greater than 18 inches below soil surface.

(iii) *Chemical foliage sprays.*—Arsenic pentoxide was found to be the most effective chemical spray, and work was concentrated on this chemical. It was found that 93 per cent. of hoary cress could be eradicated for a period of twelve months after treatment. The success of the treatment depended on:—

(*a*) The amount applied. A 6 per cent. solution at the rate of 120 gallons per acre was found to be most satisfactory.

(*b*) A rainless period of at least one week immediately prior to spraying.

(*c*) Dry soil conditions at the time of spraying.

(*d*) The spray should be applied at flowering.

(iv) *Cultivation.*—Under Werribee conditions, and using a small scarifier fitted with a weed knife, hoary cress could be *completely* eradicated if the intervals between cultivations did not exceed fourteen days, and cultivations were *regularly* carried out for a period of not less than two years.

This method would be entirely unpractical over large areas on a Wimmera farm, as 50 regular cultivations in two years without a crop return would be uneconomic. It may have a limited use on small patches of the weed.

Probably the first experiments in Australia aimed at controlling hoary cress were those laid down by the Victorian Department of Crown Lands and Survey. The experiments were commenced in 1926 on three acres of badly infested cress land in the Wimmera, and treatments were continued until 1931. It was hoped to find an economic and practical method of eradicating cress, and by 1931 observation showed salting to be quite effective, and regular cultivation at 14-day intervals to be quite efficient though costly.

The plots were inspected again in 1939, and, although eight years had elapsed since any treatments were applied, the effects of some treatments were very pronounced, and it was considered desirable to obtain quantitative results, not only of the treatment effect in reducing cress, but also its effect in increasing or decreasing the growth of species suitable for pasture.

For this purpose, counts were made in October 1939 of the number of hoary cress plants present in each plot, as represented by the mean number of plants in each of ten casts of a 4-square-link quadrat. Pasture samples were cut from each plot, the edible species were separated from the hoary cress, and the whole was oven-dried and weighed; the amounts of hoary cress and of edible herbage produced per acre were then calculated. In addition, an estimate was made on each plot of the amount of ground covered by hoary cress, legumes, grasses, "other species," and the proportion of bare ground. From these figures a picture can be formed of the appearance of the pasture growth on each plot.

The herbage species present comprised:—Wimmera rye-grass (*Lolium* spp.); soft brome (*Bromus mollis*); barley grass (*Hordeum murinum*); Burr medic (*Medicago denticulata*); *Medicago lupulina*; and such other species as dandelion (*Hypochaeris radicata*) and milk thistle (*Sonchus oleraceus*).

It is emphasized here that these plots were unreplicated, and that it is not possible to calculate the magnitude of error in the plot results. However, the effects of the treatments are so clear cut in the field that the results probably give a very reliable indication of treatment effects.

The results are presented in Table 2. Where a number of plots received similar treatment, the mean results from those plots are presented. Plot numbers in the first column show the amount of replication.

It will readily be seen that some treatments very effectively reduced the density of hoary cress and produced a reasonable yield of herbage with a desirable balance between legumes and grasses. Arsenic trioxide and Weedex, no treatment, cultivation, sulphuric acid and straw thatch, and sulphuric acid without thatch, are treatments which have reduced cress density to less than five plants per 4 square links. Of these, the arsenic trioxide and Weedex treatment must be deleted, for a glance at the other columns shows that it reduced cover to little other than the few plants of hoary cress (see Pl. 3, Fig. 1).

A total herbage yield of more than 11 cwt. per acre is produced on plots given the following treatments:—Arzeen, cultivation, carbon bisulphide, calcium chlorate and Weedex, no treatment, sulphuric acid without thatch, arsenic trioxide with thatch, and sulphuric acid with thatch. The treatments Arzeen, carbon bisulphide, calcium chlorate and Weedex, cultivation, and arsenic trioxide and thatch, should be deleted because of their high cost or impracticability; but even excluding this factor, all except the last two have an excessive proportion of bare ground, due to the poisonous effect of the treatments, and also a great amount of the herbage produced is made up of the inedible hoary cress itself or of relatively innutritious plants (see Pl. 3, Fig. 2). On the Arzeen plot, for instance, 11.44 cwt. of herbage is produced. Almost two-thirds of this is the inedible hoary cress, the plant which is to be eradicated; one-third of the remaining edible material is dandelion, and 64 per cent. of the plot area is bare ground, so poisoned that neither hoary cress nor palatable species will grow on it.

We are left then with cultivation (Pl. 4, Fig. 1), no treatment (Pl. 4, Fig. 2), sulphuric acid without thatch, arsenic trioxide with thatch, and sulphuric acid with thatch (Pl. 4, Fig. 3) as treatments which produce reasonable bulk of pasture with small cress contamination, for even though the number of cress plants is high in the arsenic and thatch plot the bulk of cress is low. Incidentally, it is interesting to see that the highest yields are from plots which obtained organic matter in the form of straw thatch—an indication of reduced fertility after a wheat fallow rotation. There is considerable difference in the pastures produced on them after each of these treatments, because, in the arsenic and straw-thatch plots, legumes cover only one-twelfth of the ground covered by grasses, while in the sulphuric acid plots with straw-thatch grasses cover five times as much ground as the clover-like plants. This, of course, is to be expected in plots which have been thatched, for organic matter will depress the legumes and increase the grasses. Sulphuric acid without thatch, cultivation, and no treatment,

TABLE 2.—DENSITY OF HOARY CRESS, PERCENTAGE GROUND COVER, AND TOTAL HERBAGE PRODUCED IN 1939.

Plot Nos.	Treatment.	Density of Hoary Cress—Plants per 4 sq. lks.	Estimated percentage ground cover.					Yield of herbage expressed as cwt. of oven-dry herbage per acre.		
			Hoary cress.	Legumes.	Grasses.	Other species.	Bare ground.	Hoary cress.	Edible species.	Total.
3A ..	*Salt and straw thatch*— Three tons salt per acre covered with 9-inch straw thatch, May 1926; sulphuric acid spray, 1928, 1929: sodium arsenite spray, 1930, 1931	27·8	16·3	11·1	22·2	5·2	45·2	2·9	7·24	10·14
3B, 3C, 3D, 3E, and 4F	*Salt, no thatch*— Mean total application of salt per acre between 1926 and 1931 equals 12.5 tons	16·04	9·74	5·62	18·58	0·68	65·38	2·86	6·63	9·49
4A.1 ..	*Arsenic trioxide and straw thatch*— Arsenic applied as sodium arsenite. 1½ cwt. arsenic trioxide covered by 9-inch straw thatch, May, 1926; 5 cwt. arsenic trioxide from 1928 to 1931	25·0	12·6	4·5	57·7	2·9	22·3	0·12	24·6	24·72
4A.2 ..	*Arsenic trioxide and Weedex*— Arsenic applied as sodium arsenite. 8 cwt. arsenic trioxide, May, 1926, to May, 1928; 3 cwt. Weedex, October, 1928	4·9	0·7	..	..	..	99·3	0·5	0·08	0·58
4B.1 ..	*Arsenic trioxide (applied as sodium arsenite), no thatch*— 12 cwt. arsenic trioxide per acre between May, 1926, and October, 1931	25·5	13·2	1·0	16·4	2·5	66·9	1·51	7·16	8·67
1aA ..	*Calcium Chlorate*— 4 cwt. calcium chlorate per acre, November, 1936	23·8	23·8	0·8	0·4	..	75·0	..	..	..
1aC2 ..	*Calcium Chlorate and Weedex*— 4 cwt. calcium chlorate per acre, May, 1936; 4 cwt. Weedex per acre, 1930–31	23·0	18·0	4·8	20·8	13·8	42·6	1·26	14·1	15·36

TABLE 2—*continued*.

Plot Nos.	Treatment.	Density of Hoary Cress—Plants per 4 sq. lks.	Estimated percentage ground cover.					Yield of herbage expressed as cwt. of oven-dry herbage per acre.		
			Hoary cress.	Legumes.	Grasses.	Other species.	Bare ground.	Hoary cress.	Edible species.	Total.
1bA1, 1aC2, 4B2	*Carbon bi-sulphide*— 118 gals. carbon bi-sulphide per acre ..	23·53	12·2	1·5	15·8	14·3	56·2	3·20	9·56	12·75
1bA2, 1bB, 5D	*Arzeen (80 per cent. Arsenic trioxide)*— Mean application of 5 cwt. Arzeen per acre in one season	21·3	17·9	3·6	9·6	4·6	64·3	7·34	4·1	11·44
1aD, 4C, 1bC, 5C, 6D	No treatment	4·86	1·0	41·16	42·47	0·4	14·97	0·12	17·58	17·70
2 ..	*Cultivation*— Cultivated weekly for 12 months, and fortnightly for 18 months. Sown to wheat 1932, and yielded 14 bags per acre	4·4	1·4	45·9	35·9	0·2	17·6	0·15	11·8	11·95
5A ..	*Sulphuric Acid and straw thatch* (480 gals.)— 10 per cent. acid, May, 1926, followed by 9-inch straw thatch. 8 per cent. acid, 1927; 6 per cent. acid 1928, 1929, 1930, and 1931, at 480 gals. per acre	4·1	1·3	14·1	67·5	0·3	17·9	0·05	28·0	28·05
5B ..	*Sulphuric Acid, no thatch*— 10 per cent. acid, May, 1926; 8 per cent. acid, May and October, 1927; 6 per cent acid, October, 1928, 1929, 1930, and 1931, at 480 gals. per acre	2·4	0·7	46·5	37·9	0·2	14·7	0·09	20·13	20·22

plots show a very desirable balance of grasses and clovers, and doubtless yields would have been much greater had the plots been top-dressed with superphosphate each year and judiciously grazed.

Some explanation must be made of the results obtained with salt treatments in these plots. In 1931, when treatments ceased, the salt-treated plots were free of vegetation, and salt appeared, under those conditions, to be the cheapest and most effective treatment for eradicating hoary cress. It was, therefore, recommended for eradicating small patches of cress, for although it left the soil sterile for some years, it was quite a good insurance against spreading the weed over the whole farm. Many farmers claim considerable success by this method, while others state that they have not had such good results. It is probable that those gaining success have begun the treatment before the weed has fully developed its root system, or have carried the work out more thoroughly. On this particular series of plots, set down in well-established hoary cress, salt has not kept the cress down eight years after treatment ceased. Further, salting in the Wimmera causes a very obvious change in the clay complex. It is to be expected in such soils, containing between 40 and 50 per cent. of clay and some free calcium carbonate, that an easily puddled, toxic sodium clay should develop, and with only a 16 to 18-in. rainfall, and no irrigation water available, it must be many more years before those soils return to normal, even if they ever do (see Pl. 3, Fig. 3).

5. Discussion.

Excepting the arsenic with straw-thatch treatment and the cultivation treatment because they are costly and not practicable over large areas, the cheapest treatments appear the most satisfactory after the lapse of ten years. The high yields from the acid-treated plots is possibly brought about by a slight change in the pH (acidity) of the soil. It is probable that a similar change would be brought about, and perhaps more rapidly, by liberal top-dressing with superphosphate and judicious grazing, and an increased yield from the no treatment plot would be obtained by similar treatment.

It is now becoming accepted in Australia (and these results point to the same conclusion) that deep-rooted, perennial weeds on wheat lands with a rainfall of 16 to 20 inches can only be successfully combatted by extending the rotation to include a period of sown pastures to be grazed by sheep. It has been pointed out that hoary cress becomes a serious pest only under cultivation associated with wheat farming, and that Wimmera soils do show signs of depletion after many years of wheat farming. It is logical, then, to assume that a considerable measure of control may be obtained by altering the system of farming. The development of suitable pastures and management technique for the Wimmera therefore is of prime importance. At Werribee, success in controlling hoary cress has been achieved for many years with irrigated lucerne pastures, and similar success has recently been obtained in the United States of America.

With the more complete knowledge of factors influencing chemical foliage spray efficiency, there is hope of using that method for eradicating smaller patches of cress and of continuing Morgan's work, while soil sterilants may be useful if used early and if their effect on Wimmera soils can be made less permanent.

A Study of the Alkaline Extraction of Wood.

1. The Effects of Hot Alkaline Treatments on *E. regnans* F. v. M.

*By A. W. Mackney, M.Sc., A.A.C.I.**

Summary.

A detailed analytical study has been carried out using samples of *E. regnans*, extracted with dilute sodium hydroxide solutions of various concentrations.

It has been shown that for this species a satisfactory preliminary to the lignin determination is extraction with just sufficient sodium hydroxide to maintain an alkaline reaction at the end of the treatment period. It is indicated that, in previous investigations of the use of sodium hydroxide as a pretreatment in the lignin determination, the alkaline treatments applied have been unnecessarily drastic, at least for woods similar to *E. regnans*.

Evidence has been produced to show that the lignin of this species consists of two fractions, the larger containing 24.4 per cent. methoxyl and being resistant in situ to hot sodium hydroxide solutions up to 2 per cent. concentration, the smaller containing 20.6 per cent. methoxyl and being removed from the wood by sodium hydroxide solutions of concentrations greater than 0.08 per cent.

The conditions governing the removal of the fibre cementing material have been determined, and considerable analytical data concerning this fraction, which constitutes approximately 5 per cent. of the wood, are given. It is shown that this material, which initially remains in the holocellulose fraction, is a non-furfural yielding, non-carboxyl, and non-methoxyl bearing body.

In addition to the fibre cementing material, there is present in holocellulose a non-cellulosic non-furfural yielding carbohydrate to the extent of approximately 5 per cent.

The constitution and yield of Cross and Bevan cellulose are shown to vary with the nature of the alkali pretreatment. Up to a certain point there is an increase in cellulose with a corresponding increase in xylan in cellulose. With more severe treatments the cellulose yield decreases, but the xylan in cellulose remains significantly higher than its initial value. An attempt is made to explain these variations.

1. Introduction.

Although the alkaline treatment of wood samples has been utilized for various chemical investigations over a period of years, little systematic work in the field appears to have been attempted, and no detailed information is available as to the nature of the constituents of wood that are removed or modified by this reagent. The present study may be regarded as introductory to a number of more detailed investigations, particularly in regard to the question of the composition of the total carbohydrate fraction of wood; it provides considerable information regarding three important determinations, viz.: lignin, holocellulose, and Cross and Bevan cellulose.

The suggestion was put forward by Cohen (1, 2, 3) that a pretreatment with 0.5 per cent. sodium hydroxide should be applied for the purpose of removing extraneous materials which are insoluble in the usual organic solvents and water, and which interfere in the lignin determination. Objection to this treatment was raised by

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Harris (4) who contended that the alkali also removed some lignin. However, the sodium hydroxide extractions carried out by the latter were unnecessarily severe and additional data are required to clarify the point. This is made more desirable through the discovery by Cohen and Harris (5) that there appears to be present in lignin a major fraction of stable insoluble material accompanied by a smaller amount of a more soluble form which is relatively easily removed by certain chemical treatments. It is necessary to decide whether lignin is to be defined as only the resistant portion or should include also the less stable smaller fraction.

Recent work on the cell wall and the intercellular layer has led to the discovery by Cohen and Dadswell (6) that, when a mild alkaline pretreatment at boiling temperatures is followed by chlorination and a final cold alkali treatment, sections or thin shavings of wood will break up readily into fibres. A pulp is obtained in yields of 66 to 69 per cent. and retains 85 to 90 per cent. of the furfural-yielding constituents of the original wood. It is clear from this work that the alkaline pretreatment has the effect of removing or modifying some non-lignin material concerned in the bonding together of the wood fibres, while the remainder of the carbohydrate constituents are little affected. Controlled delignification, as in the preparation of holocellulose (7), does not cause maceration even when all the lignin is removed, but, when delignification is followed by mild alkaline treatment, maceration occurs readily. Hence it is clear that an investigation of controlled mild alkaline treatments may give some indication of the constitution of holocellulose, and, in particular, of that portion concerned in the bonding together of fibres.

Finally, it has been observed (unpublished work) that, although treatment of a wood sample with hot alkaline solutions has the effect of removing furfural-yielding constituents, there is at the same time a distinct increase in the percentage of Cross and Bevan cellulose. This increase is almost wholly accounted for by an increase in the percentage of furfural-yielding constituents of the cellulose. Also, when pulping is carried out with an excessive quantity of alkali, there is an increase in furfural-yielding constituents over and above that for a normal pulp*. It was hoped that systematic study of alkaline extractions would throw some light on this problem and indicate just what fraction is to be considered as Cross and Bevan cellulose.

With regard to terminology of the carbohydrates of wood, it is abundantly clear that there is considerable confusion regarding the application of the term "polyuronide" which has been used loosely with little indication of a definite meaning. In order to distinguish from polyuronides of the nature of pectins and gums, the term polyuronide-hemicellulose has been used by Norman (8) to indicate a pentose (or hexose) + uronic acid, or pentose + hexose + uronic acid complex. In addition, Norman (8) has recognized the division of the alkaline extract into two distinct types, viz.: (*a*) an amorphous encrusting material not associated with cellulose, and (*b*) a smaller amount derived from the cellulosan fraction of natural cellulose. In

* This point was raised in a previous publication (Cavanagh, J. C., Dadswell, H. E., Mackney, A. W., and Reynolds, T. M.—Division of Forest Products, Tech. Paper No. 29, Coun. Sci. Ind. Res. (Aust.), Pamph. No. 86, 1938), but no solution of the anomaly was discovered.

this work such a distinction has been observed but it should be pointed out that, from the results of the cellulose determination, a rigid differentiation between cell wall and encrusting material is difficult to maintain.

2. Experimental.

The material used was prepared from the butt portion of a mature log of *E. regnans* by disintegration in a Wiley mill. The portion selected was that fraction which passed through a 60-mesh sieve and was retained by an 80-mesh sieve. The major portion of the extractives was removed by treating bulk quantities of 300 g., in four batches of 75 g. each, in Mason jars which were clamped in a bleach tank and tumbled. Each portion was treated three times with water at 20°C. (1,250 ml. water per 75 g. wood), the periods being 90, 45, and 30 minutes. After each water extraction the material was filtered on large Buchner funnels and washed with water. After the third water treatment the material was washed with 81 per cent. alcohol until colourless, then tumbled for two periods (60 min., 30 min.) with 84 per cent. alcohol at 20°C. (1,000 ml. per 75 g. wood). It was then filtered and washed with 84 per cent. alcohol, and refluxed with stirring for 30 minutes with 99 per cent. alcohol (1,000 ml. per 75 g. wood). It was finally filtered and washed with alcohol till the washings were colourless, sucked dry, and placed in a flat dish to come to equilibrium with the atmosphere. The amount extracted by this treatment was 4.7 per cent. of the untreated wood.

To prepare bulk quantities of alkali extracted material, eighteen samples, each approximately 3 g. in weight, were weighed accurately. Sodium hydroxide solution of the required strength was prepared and tested by titration against standard acid. The required volume of alkali calculated on the oven-dry weight of the wood was added to each, and the samples were extracted for one hour in the boiling water bath at approximately 98°C. They were then filtered on R.A. 98 alundum crucibles, and washed with 1,500 ml. hot water. Three samples chosen at random were oven-dried to determine the amount extracted. The remaining fifteen samples were transferred to a large flat dish, thoroughly mixed, and allowed to stand for some days to come to equilibrium with the atmosphere. To determine the percentage of sodium hydroxide consumed during the extraction, the filtrate and first washings from three samples were collected. These were made up to 500 ml., 25 ml. aliquots were taken, diluted with 100 ml. water, and titrated with standard acid of a suitable concentration.

Fourteen extracted samples were prepared in this way and these, together with the original solvent treated sample, were submitted to the following analyses:—Lignin (9), total furfural (10), C. and B. cellulose, furfural in cellulose (10), total carbohydrate (holocellulose), furfural in total carbohydrate (10), carboxyl groups, total methoxyl, methoxyl in lignin, methoxyl in total carbohydrate (11), copper number, and permanganate number of total carbohydrate (12). In certain cases the nitrogen content of total carbohydrate was determined by the Kjeldahl method.

The following notes on the methods are appended:—The lignin determination was carried out by the U.S. Forest Products Laboratory method, but at the completion of the wash with 1,000 ml. water the

crucible was filled with water and placed in a small beaker on the boiling water bath for a period of at least 30 minutes. It was then washed further with 300 to 500 ml. hot water.

Furfural was determined using the Tollens' distillation followed by precipitation with thiobarbituric acid. Furfural was calculated from the equation given by Mackney and Reynolds (10) and conversions to xylan were made using Kröber's factor. It is recognized that calculation of all furfural as xylan involves an error due to neglect of furfural obtained from uronic acids, but since the percentage of uronic acid is small the total error is not great.

C. and B. cellulose was determined by a chlorine water method standardized in this laboratory (unpublished work) from the method described by Benjamin and Somerville (13).

Total carbohydrate (holocellulose) was determined by alternate treatments with chlorine water and cold alcoholic ammonia (10 ml. ammonia (Sp. G. 0.880) and 90 ml. 99 per cent. alcohol)*. Seven chlorinations were carried out with a final water wash for 30 minutes in the shaker. The first chlorination was carried out in a beaker and the first two alcoholic-ammonia extractions in the shaker; otherwise all treatments were applied in the crucible.

Carboxyl groups were determined using Campbell's apparatus and Campbell's modification (14) of the method of Dickson, Otterson, and Link (15).

Weighings for the semi-micro methoxyl determinations were carried out on a Sartorius air-damped balance. In the case of methoxyl in lignin, the determination was carried out on samples which had been allowed to dry at room temperature.

Copper number was determined by the TAPPI tentative standard method (16) with the modification that ferric ammonium sulphate and sulphuric acid were used for dissolving the cuprous oxide in place of molybdophosphoric acid.

The results of the above analyses on the fifteen samples are set out in Tables 1 and 2 as required for the discussion.

In addition, bulk C. and B. cellulose samples were prepared after pretreatments corresponding to those given to samples 0, 8, and 13 in Table 1. These bulk samples were analysed, the determinations carried out being furfural, uronic acid, copper number, and alpha-cellulose. The methods were those already described above, with the addition that the alpha-cellulose determination was carried out on 1 gram samples treated for 45 minutes with 17.5 per cent. sodium hydroxide. The results of these analyses are given in Table 3.

3. Discussion.

(a) *General Considerations.*—By reference to Table 1 it can be seen that for samples 1 to 5, in the extraction of which there was present either insufficient alkali or only a slight excess, the amount extracted is a function of the total amount of alkali available rather than of the concentration of the solution. This is shown by the fact that, although sample 5 was extracted by an alkaline solution having twice the

* Kerr and Bailey (22) originally used this method for the complete removal of lignin from wood sections. The method has been modified in this laboratory (unpublished work) to be applicable to analytical studies.

concentration of that used for sample 3, there is little difference between the amounts removed. On the other hand, samples 6 to 14 were extracted by a considerable excess of alkali and the amount extracted is a function of the concentration of the solution. This is shown by samples 12 and 13 which are practically identical, even though sample 13 was extracted with 50 per cent. of available alkali as compared with 25 per cent. for sample 12, each solution having a concentration of 1 per cent. An exception is shown by samples 8 and 9 for which the same solution concentration was used (0.16 per cent.) but considerably more was extracted from the latter. In this case apparently a threefold increase in available alkali was sufficient to cause more thorough extraction.

It is clear that the consumption of alkali is a function of the amount available and no upper limit has been reached in this series even with sample 14. This fact indicates the difficulty of attempting to outline conditions for the determination of alkali consumption, this quantity being defined rather by the amount available than by the state of the wood.

As a general indication of the state of the wood after extraction, the copper number determination was carried out on each of the fifteen samples. The results (Table 1) show no evidence of degradation through the action of the stronger solutions of sodium hydroxide. After the removal of extraneous material from sample 0 there is a very pronounced drop in the value, but thereafter there is only a steady decrease throughout the series.

TABLE 1.*

Sample No.	Sodium Hydroxide Percentage Wood.	Sodium Hydroxide Percentage Solution.	Sodium Hydroxide Consumed Percentage Wood.	Extracted by Sodium Hydroxide Percentage Wood.	Copper No.	Lignin.	Methoxyl in Lignin.	
							Percentage Lignin.	Percentage Wood.
0	..	..	..	..	5·5	22·9	22·90	5·25
1	1·25	0·013	1·3	2·57	2·3	21·6	23·70	5·14
2	1·75	0·027	1·8	3·41	2·2	21·5	23·84	5·14
3	2·5	0·027	1·9	4·15	2·3	21·5	24·14	5·19
4	2·5	0·040	2·1	4·07	2·2	21·7	24·09	5·23
5	2·5	0·053	2·2	4·39	2·1	21·8	23·98	5·22
6	5·0	0·053	3·4	5·92	1·9	21·7	23·87	5·19
7	5·0	0·08	3·7	6·94	1·8	21·5	23·97	5·15
8	5·0	0·16	3·8	8·06	1·9	20·9	24·16	5·05
9	15·0	0·16	4·8	9·29	1·7	20·4	24·34	4·98
10	15·0	0·32	5·2	10·87	1·6	19·8	24·62	4·87
11	15·0	0·48	5·6	11·39	1·6	19·9	24·45	4·86
12	25·0	1·0	6·3	12·96	1·6	19·9	24·38	4·85
13	50·0	1·0	6·8	12·97	1·5	19·9	24·23	4·81
14	100·0	2·0	8·4	14·12	1·4	19·8	24·47	4·85

* Analyses, except where otherwise stated, are per cent. of the original solvent extracted sample 0.

(*b*) *Lignin.*—The lignin value and methoxyl in lignin of the fifteen samples are reported in Table 1. In addition, the following figures were determined for the completely untreated wood, i.e., prior to solvent treatment:—

Lignin = 24.61 per cent.
Methoxyl in Lignin = 20.16 per cent. lignin.
= 4.96 per cent. wood.

Since solvent extraction of the wood removed 4.7 per cent., this methoxyl in lignin figure corresponds to 5.21 per cent. of the solvent extracted wood.

It is clear that from samples 0 to 7 the methoxyl in lignin expressed as a percentage of the wood remains constant, and, if loss in methoxyl may be taken as a criterion of loss in lignin, there has, up to this point (extraction with 5.0 per cent. sodium hydroxide at 0.08 per cent. concentration) been no removal of lignin. In addition, the extraneous material which inflates the lignin values for the unextracted sample and sample 0 does not contain methoxyl as shown by the constancy of the methoxyl in lignin (per cent. wood) during its removal.

It remains to decide whether extraneous material has been entirely removed throughout this portion of the series. The lignin values from samples 1–7 are virtually constant with an average value of 21.61 per cent. and the methoxyl expressed as percentage of lignin also remains constant at 24.0 per cent. (with the possible exception of sample 1). Hence it can be assumed that this is a true lignin figure containing the whole of the original methoxyl in lignin and not contaminated with extraneous material.

The extraction as carried out for samples 8 and 9 causes an immediate decrease in the lignin value until at sample 10 it again becomes constant and remains so even after the most drastic treatments applied. That this is an actual removal of lignin is clear from the fact that the methoxyl in lignin, calculated as a percentage of the wood, decreases from 5.20 per cent. to 4.85 per cent. There is, however, at the same time a slight increase in the methoxyl (expressed as percentage of lignin) from 24.0 to 24.4 per cent. It appears that in this wood sample there is 19.8 per cent. of a lignin which is resistant to the action of boiling alkali in concentrations as high as 2.0 per cent., together with approximately 1.8 per cent. of a less resistant type of lignin which, however, is only stable to boiling alkali up to 0.08 per cent.

The methoxyl content of the more stable form is 24.4 per cent., which amounts to 4.83 per cent. methoxyl in the wood sample. The average methoxyl content of the two forms is 24.0 per cent. with a total methoxyl amounting to 5.20 per cent. of the wood. Hence it may be calculated that the smaller quantity of less stable lignin has a methoxyl content of approximately 20.6 per cent. It can be seen clearly that there is some considerable difference between these two forms; nevertheless it is contended that the true lignin is the sum of the two values and not the residue which is higher in methoxyl and remains after more drastic treatment. This suggestion is based on holocellulose figures which will be discussed later, and on the application of the formula derived by Cohen (3) to give the true lignin content of wood. This is applied as follows:—

$$\text{Lignin content} = \frac{5\cdot 25}{24\cdot 45} \times 100$$
$$= 21.5 \text{ per cent. (approximate)}$$

where 5.25 is the methoxyl in lignin as per cent. of the wood after extraction with organic solvents, and 24.45 is the per cent. of methoxyl in lignin after extraction with 0.5 per cent. sodium hydroxide.

This figure is in close agreement with the average for samples 1–7.

A convenient standard for pretreatment for the lignin determination for this species would be that the solvent extracted sample be treated with an amount of alkali sufficient to provide a slight excess, the concentration of the alkaline solution being kept at 0.08 per cent. or lower. It must be emphasized that this finding applies only to this species, and the desirability of standardized sodium hydroxide pretreatments for lignin determinations on all species is not suggested. Nevertheless, there is some evidence to show that in other species the lignin may consist of a resistant portion together with a smaller fraction which is more or less affected by the chemical pretreatment applied. Thus Cohen and Harris (5) found that treatment of maple wood with 3 per cent. sulphuric acid reduced the lignin figure to a constant level at 17.7 per cent. after removing 4 to 5 per cent. of so-called "soluble lignin." In this case it was suggested that the soluble lignin was identical with methanol lignin, but was retained during the lignin determination through polymerisation caused by 72 per cent. sulphuric acid. The amount of soluble lignin in maple is evidently considerably greater than that in *E. regnans* and emphasizes clearly that the true lignin figure must include this fraction.

An investigation carried out by Harris (4) regarding the effect of alkali treatment on the yield of lignin from maple must be discussed in connexion with this work. It is not believed that the results obtained in the present investigation can be applied to maple, but several observations may be made. The alkali treatments carried out by Harris involved the use of a series of solutions of increasing concentration, but the minimum concentration used was 0.5 per cent., which, on a liquor ratio of 200/1, represents 100 per cent. alkali available to the wood. The series extends through a range up to 10 per cent. solution which, with the same liquor ratio, is an enormous excess. These treatments were uniformly unnecessarily vigorous and it is quite probable that even the more resistant form of lignin was removed. Pretreatment with alkali for the lignin determination aims at removal of extraneous material which acts readily with alkali and therefore an amount sufficient to maintain a slight alkaline reaction at the end of the extraction is all that is required.

Previous work published from this laboratory (1, 2, 3) has employed as standard pretreatment for the lignin determination an extraction with 0.5 per cent. sodium hydroxide, which, at a liquor ratio of 100/1, represents about 50 per cent. of alkali available to the wood. If the above results are applicable this treatment has been too drastic for woods of the type used in this study and at best the lignin value obtained will represent the resistant fraction, although it has been demonstrated (3) that the lignin removed may be recovered by acetic acid precipitation.

One further point should be emphasized in connexion with this determination. It has been stated in the experimental section that the lignin determination was carried out according to the modified United States Forest Products Laboratory method (9) and, in addition, an improved washing technique was adopted. The importance of this can be seen in the fact that samples prepared without the additional wash, although agreeing closely in their total lignin values, were 1.0 to 1.3 per cent. lower in methoxyl in lignin than those prepared subsequently by the modified technique. This apparently is due to retention of

traces of sulphuric acid by the lignin when not completely washed, and this, although not sufficient to affect the total yields, may cause some demethylation. The effect was not in this case large enough to be detected in the lignin determination, but was shown clearly in the methoxyl in lignin.

(c) *Holocellulose.*—Since the early work of Schmidt (17) in 1931 for the preparation of "skelettsubstanzen" was extended by Ritter and co-workers (7, 18, 19, 20, 21) to provide an easily applied method for determining "holocellulose" or the "total carbohydrate fraction of wood", it has become possible to determine this fraction on a routine basis. However, when it was attempted to apply the method to eucalypts, difficulty was encountered (unpublished work) mainly because of the indefinite end-point and the difficulty of controlling gaseous chlorination. In view of preliminary results from an investigation which is not yet complete, the method as outlined in the experimental section was adopted. No claim is made for the superiority of this method except that in using chlorine water and cold solvents it is much more readily adopted by a routine worker. Indeed it is not possible by this method to determine an accurate end-point so that an arbitrary number of chlorinations has been adhered to throughout. Rather, the aim of the work has been that, through rigid standardization, comparable results might be achieved throughout the series and variations in holocellulose observed. It is probable that the fraction isolated as holocellulose contains a trace of lignin but this would not amount to more than 0.5 per cent. and would, in most cases, be less. However, at least in the case of samples 0, 1, and 2 (Table 2), there is no loss of carbohydrate material (other than that removed by the alkali extraction), as shown by the agreement between total xylan and xylan in cellulose, and by the fact that the sum of extractives, holocellulose, and lignin is slightly over 100 per cent. In the case of sample 0 the sum of holocellulose and lignin (102.4 per cent.) is inflated by extraneous material which is estimated in both holocellulose and lignin determinations. The more accurate figure is obtained by summing holocellulose and true lignin (21.5 per cent.) which makes a total of 101.0 per cent.

Reference to Table 2 shows that there is in no case a significant loss of furfural-yielding materials during the holocellulose determination. Throughout the series there is no significant difference between total furfural and furfural in holocellulose (columns 3 and 5), with the possible exception of sample 7 where there is a discrepancy of 0.4 per cent. This difference may be due to an accumulation of experimental errors or possibly to the presence of a small amount of degraded xylan in the original wood sample, which is removed during later chlorination and alkaline extraction. Hence it is clear that apart from furfural-yielding materials removed in the alkaline extraction, all such remaining after extraction are retained in the holocellulose fraction.

Examination of column 12, giving the sum of sodium hydroxide extractives, lignin, and holocellulose, shows that in the case of samples 3, 4, and 5 there is a slight discrepancy between the value obtained and the theoretical 100 per cent. This must indicate that the nature of the alkali treatment is such as to modify some portion of the carbohydrate material rendering it soluble in the subsequent chlorination-extraction

procedure. For samples 6, 7, and 8 this effect is much more pronounced as the alkali concentration (0.05 to 0.16 per cent.) becomes sufficient to modify the whole of this material and permit its removal during subsequent treatment. From sample 8 to 14 the sum shown in column 12 remains constant at approximately 95.7 per cent., and it is clear that there is approximately 5 per cent. of a material present in the original wood whose properties differ markedly from those of the remainder of the carbohydrate fraction.

TABLE 2.*

Sample No.	Sodium Hydroxide Percentage Solution.	Extracted by Sodium Hydroxide Percentage Wood.	Total Furfural.†	Holo-cellulose.	Furfural in Holo-cellulose.†	Permanganate No. of Holo-cellulose.	Methoxyl in Holo-cellulose.	Total Methoxyl.
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
0	..	..	11·6	79·5	11·6	10·3	1·24	7·40
1	0·013	2·57	11·4	75·8	11·3	8·5	0·96	7·24
2	0·027	3·41	11·3	75·8	11·2	8·7	0·93	7·13
3	0·027	4·15	11·2	73·8	11·1	7·8	0·82	7·11
4	0·040	4·07	11·2	73·8	11·3	7·9	0·86	7·12
5	0·053	4·39	11·1	73·5	11·2	8·0	0·94	7·12
6	0·053	5·92	11·1	69·9	10·9	4·8	0·66	7·00
7	0·08	6·94	11·0	67·7	10·6	3·6	0·54	6·97
8	0·16	8·06	10·7	66·3	10·6	2·8	0·48	6·81
9	0·16	9·29	10·4	66·2	10·5	3·0	0·48	6·57
10	0·32	10·87	10·1	65·1	10·2	2·4	0·44	6·56
11	0·48	11·39	10·0	64·0	10·0	2·1	0·40	6·59
12	1·0	12·96	9·7	62·8	9·5	1·6	0·35	6·33
13	1·0	12·97	9·6	63·2	9·6	1·9	0·41	6·40
14	2·0	14·12	9·1	61·7	9·0	1·7	0·37	6·43

Sample No.	C. and B. Cellulose.	Furfural in C. and B. Cellulose.†	Uronic Acids.	Sum of Sodium Hydroxide Solubles, Lignin and Holo-cellulose.	Methoxyl not in Lignin and Holo-cellulose.	Xylan not in Cellulose.	Carbo-hydrate other than C. and B. cellulose or xylan.	Nitrogen (Percentage Holo-cellulose.) §
	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
0	61·4	8·1	4·70	102·4	0·99	5·4	12·7	0·66
1	61·2	8·1	4·20	100·3	1·14	5·2	9·4	0·50
2	61·6	8·2	3·93	100·8	1·06	4·7	9·5	0·37
3	61·3	8·1	3·78	99·4	1·10	4·8	7·7	..
4	61·9	8·5	3·87	99·6	1·03	4·2	7·7	..
5	62·0	8·4	3·81	99·7	0·96	4·2	7·3	0·38
6	62·3	8·9	3·63	97·5	1·15	3·4	4·2	..
7	62·5	9·1	3·59	96·1	1·28	3·0	2·2	..
8	63·0	9·2	3·20	95·3	1·28	2·3	1·0	0·10
9	61·7	8·6	3·11	95·9	1·11	2·8	1·7	..
10	61·5	8·7	2·90	95·8	1·25	2·1	1·5	0·08
11	60·8	8·5	2·88	95·3	1·33	2·4	0·8	..
12	59·9	8·3	2·79	95·7	1·13	2·1	0·8	..
13	60·2	8·5	2·73	96·1	1·18	1·8	1·2	0·07
14	59·4	8·3	2·60	95·6	1·21	1·3	1·0	0·07

* Analyses, except where otherwise stated, are per cent. of the original solvent extracted sample 0.

† Furfural figures may be converted to xylan by multiplying by Krober's factor (viz., 1·552).

§ The nitrogen content of the original wood (sample 0) was 0·07 per cent.

From the analytical figures available, and from the fact that this fraction is present in all wood samples and in holocellulose samples 1 to 5, but absent from holocellulose samples 8 to 14, certain important facts may be deduced. Primarily, it is non-furfural yielding since there is close agreement between total furfural and furfural in holocellulose. This eliminates the possibility of it being predominantly either a pentosan or polyuronide material, since even 5 per cent. of the latter would be expected to yield 1 per cent. of furfural. Also, further evidence against the possibility of a polyuronide structure is obtained somewhat indirectly from the evidence of uronic acid values given in Table 3. It is clear that this material is still retained in the extracted wood of sample 14, since otherwise the discrepancy of 5 per cent. would not appear in column 12, Table 2. Also there is little doubt that this fraction has been removed from the C. and B. cellulose 13 in Table 3. Since there is no real difference in uronic acid values obtained for these two materials (viz., 2.6 and 2.4 per cent.), it must be concluded that the 5 per cent. fraction is non-carboxyl bearing.

Secondly, determination of methoxyl in holocellulose shows that the total amount of methoxyl in the original wood cannot be accounted for by the sum of methoxyl in lignin and methoxyl in holocellulose. There is a slight discrepancy calculated in column 13 which may be due to the presence of a small amount of unstable methoxyl removed during the acid treatments of either the lignin or holocellulose determinations. The main fact emerges that this discrepancy remains approximately constant throughout the whole series although the 5 per cent. fraction is removed, so that this material is non-methoxyl bearing.

Thirdly, examination of the permanganate number (column 6) which was determined on holocellulose shows that apart from a drop in the value from sample 0 to 1 corresponding to the removal of extraneous material, the main variation occurs between samples 5 and 8. The permanganate number of sample 8 from which the 5 per cent. fraction has been removed is just one-third of that for sample 5 which retains almost the whole of this material. Hence the substance is of such an unstable nature as to undergo ready oxidation during the brief mild conditions of the permanganate number treatment.

Finally, it is possibly of some value to note the colours of both the extracted wood samples and of the holocelluloses prepared from them. Going through the series it was observed that the colour of the wood lightened progressively with sodium hydroxide treatment up to sample 5, at which point faint yellowing appeared and increased with the severity of treatment up to sample 14, which was a distinct yellow. In the case of the holocellulose samples the reverse effect was observed. Samples 0 to 5 retained some of the appearance of the wood, being a definite creamy colour and very similar in texture to the wood. Samples 5 to 8 represented a transition point, the holocellulose improving to a good white, while samples 8 to 14 were as white as the C. and B. cellulose samples. Also samples 8 to 14 were very much more powdery than the earlier ones and consequently more difficult to handle. It seems probable, in conjunction with previous data, that the yellowing of the wood sample was due to the modification of the 5 per cent. fraction rendering it soluble during the holocellulose determination, while the improvement in the colour of the holocellulose was due to the removal of the 5 per cent. fraction.

The above facts are of outstanding significance when taken in conjunction with the observations of Cohen and Dadswell (6). These workers confirmed the observations of Kerr and Bailey (22) that delignification of wood sections does not necessarily mean that the fibres will separate and that further treatment is required to remove the bonding material which is non-lignin in nature. However, if the wood section is first treated by extraction with sodium hydroxide and then delignification is commenced, the sections will separate into fibres on stirring after the fifth stage of the treatment. It is clear that this pretreatment causes a modification of the bonding material between the fibres such as to permit its subsequent removal during the holocellulose treatment. Further, it was shown that the most effective alkali concentrations in modifying this bonding material without removing excessive amounts of carbohydrate were in the range 0.053 to 0.16 per cent. These limits are respectively those used for the extractions 5 and 8 as shown in Table 1. It may be deduced, then, that the above 5 per cent. fraction of the carbohydrate material, concerning which certain facts have been derived from the analytical data, is related to the bonding material of the fibres.

Further information on the composition of the holocellulose may be obtained by reference to column 15 of Table 2, which gives the difference between holocellulose and the sum of C. and B. cellulose and xylan not in cellulose. The latter sum is a measure of that portion of the holocellulose which is estimated by independent methods, so that the difference between the two gives a measure of material which is not C. and B. cellulose and not predominantly furfural-yielding. It has already been pointed out that the estimation of all furfural as xylan constitutes an error due to the formation of furfural from uronic acids; however, the amount of this error is small and in any case it will tend to inflate the xylan percentage and so decrease the amount of the difference shown in column 15. Hence this difference is a minimum expression of the actual amount of non-cellulose and non-pentosan material. The total value of the difference is evidently approximately 10 per cent. of the wood sample, and a large portion of this is removed between samples 5 and 8. This is the bonding material already discussed, but in addition there is another material which is gradually removed throughout the series down to sample 8. There are, then, two materials, each present to the extent of approximately 5 per cent., for which no direct methods of determination are available. One of these is alkali-soluble and is gradually removed by increasing concentrations of alkali; the other is modified by alkali and removed during the holocellulose determination. The value for uronic acids, calculated as a percentage of the wood, shows a gradual decrease throughout the series so that the alkali-soluble material is probably of a hexose + uronic acid nature.

One further point in connexion with the holocellulose determination should be noted. It was found that holocellulose isolated by either the hot or cold alcoholic-ethanolamine process or by the cold alcoholic-ammonia process retains 0.6 to 0.7 per cent. nitrogen as determined by the Kjeldahl method. This cannot be eliminated by any process which does not damage the holocellulose, and no information is available as to the nature of the grouping retained by the holocellulose. Nitrogen determinations were made on the holocellulose at certain points

throughout this series (column 16), and it was found that the nitrogen content decreased, with a major change between samples 5 and 8. From samples 8 to 14 the amount of nitrogen did not exceed that present in the original wood. Hence it seems probable that the nitrogen-bearing group is retained by the bonding material.

(*d*) *Cross and Bevan Cellulose.*—On the basis of the C. and B. cellulose determination, Norman (8, 23) has divided the hemicelluloses of wood into two definite fractions, viz.: the polyuronides or polyuronide-hemicelluloses of the encrusting material and the cellulosan retained in the C. and B. cellulose. Hence it is with some concern that it is found that the C. and B. cellulose of this species varies in both quantity and composition with the nature of the pretreatment applied. Further this variation in quantity is accounted for by variation in the cellulosan (xylan in cellulose), indicating that portion of the hemicellulose may be determined as polyuronide-hemicellulose, or as cellulosan according to the treatment applied.

Examination of the figures for C. and B. cellulose and furfural in cellulose (columns 9 and 10, Table 2) shows that each of these values increases with increasing severity of treatment up to sample 8 (5.0 per cent. sodium hydroxide to wood at 0.16 per cent. concentration). At this point the increase in cellulose is entirely accounted for by the increase in furfural in cellulose (calculated as xylan). With more severe treatments there is a decrease in both values although the losses are no longer comparable. The figure for furfural in cellulose remains throughout the series significantly higher than that for the original sample 0, whereas the cellulose value falls to 2.0 per cent. below that of the original sample. It would appear that the more severe treatments with alkali tend to attack the cellulose portion rather than the xylan associated with it.

A logical explanation of these facts is difficult to reach. Probably there is some effect arising through the breaking of a lignin-hemicellulose bond (cf. Norman (8), p. 60) by alkaline treatments of moderate severity. Prior to the rupture of such a bond it is conceivable that removal of lignin by chlorination and sulphite treatment would involve the removal of a certain amount of hemicellulose which is otherwise stable to alkali treatment. After breaking of the bond the lignin is more readily removed and the hemicellulose is retained by the cellulose as a relatively resistant fraction. In fact this material is of such stability it is retained while attack on the cellulosic material occurs.

It is difficult to associate the increase in C. and B. cellulose with decrease in lignin and removal of the fibre cementing material. Although these three inflexions occur at approximately the same point in the series there does not appear to be any definite evidence to permit the correlation of the three phenomena.

From the point of view of an analytical determination, the C. and B. cellulose procedure seems to be of diminished value in view of the above facts. The yield of cellulose will depend to some extent on the pretreatment applied to the wood sample, and it has already been pointed out that the quantity of furfural-producing constituents from a pulp may increase with increasing severity of alkali cooking. The original method as set out by Cross and Bevan required a pretreatment with hot 1 per cent. sodium hydroxide for twenty minutes, and it is

probable that this would cause some alteration in the resulting product. Undoubtedly Cross and Bevan cellulose does represent the resistant portion of the total carbohydrate of wood and is particularly useful from the point of view of pulping, but emphasis must now be laid on probable variations through the somewhat indefinite nature of the fraction.

In Table 3 are reported the results of a series of analyses carried out on bulk samples of C. and B. cellulose. These are designed to investigate whether any further important differences between these samples can be detected. The three materials are comparable with samples 0, 8, and 13 of the main series, though in each case the yield of cellulose is somewhat higher on account of a modified technique in chlorination. Similar differences in total yield and yield of xylan in cellulose are observed, but otherwise there is little variation in the cellulose. The figures for uronic acids, copper number, and alpha-cellulose are almost constant for the three samples, and there is no evidence of degradation through the alkaline treatment. Attention is also drawn to the observation of Campbell (14) that a small amount of carbon dioxide (approximately 0.5 per cent.) may be isolated from pure carbohydrates. This amount would account for the greater part of the uronic acids found in these samples, so that the figures reported may be of little significance.

TABLE 3.—ANALYSES OF CROSS AND BEVAN CELLULOSE PREPARED AFTER SODIUM HYDROXIDE PRETREATMENTS.*

Treatment Corresponds to Sample No. of Table 1.	Sodium Hydroxide Percentage Wood.	Sodium Hydroxide Percentage Solution.	C. and B. Cellulose.	Furfural in Cellulose.	Uronic Acids.	Copper No.	Alpha-Cellulose.	
							Percentage Cellulose.	Percentage Wood.
0 ..	..	..	61·8	8·3	2·54	1·2	79·0	48·8
8 ..	5·0	0·16	63·2	9·3	2·36	1·1	78·1	49·4
13 ..	50·0	1·0	61·0	9·0	2·42	0·8	79·7	48·6

* Except where otherwise stated, results are percentages of the original solvent extracted Sample O.

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Contributions to the Study of the Cell Wall.

2. An Investigation of Delignification Using Thin Cross Sections of Various Timbers.

By H. E. Dadswell, M.Sc., A.A.C.I. and Dorothie J. Ellis, B.Sc.**

Summary.

(i) Sections 18μ in thickness cut from several species were subjected to various methods of delignification, including several which are supposed to remove lignin only.

(ii) At each stage of each process sections were removed and tested for lignin by staining methods and treatment with 72 per cent. sulphuric acid according to the procedures already described.

(iii) As the delignification process proceeded there was a corresponding change in the staining reaction until finally the stain indicated complete loss of lignin; this change in the staining reaction was accompanied by a corresponding reduction in the lignin as revealed by the treatment with 72 per cent. sulphuric acid.

(iv) These results were further evidence in favour of the botanists' contention that certain staining procedures do indicate "lignified" tissue; it has been pointed out, however, that for staining work sections of standard thickness should be employed and that these should be subjected to no pretreatment of any kind.

(v) There was no definite indication that cell-wall lignin was removed before or after the lignin of the so-called "middle lamella" zone; all results pointed to simultaneous removal, although, of course, there is a much larger proportion of the lignin to be removed from the "middle lamella" zone.

(vi) When subjected to treatments employing aqueous or alcoholic sodium hydroxide, sodium sulphite, or aqueous ammonia, maceration of the sections occurred before complete delignification; on the other hand, when solvents for chlorinated lignin were alcoholic solutions of ethanolamine or ammonium hydroxide, and alcoholic washes were used at each stage, the sections did not macerate, although all the lignin had been removed.

(vii) The importance of further knowledge of the layers remaining and holding the cells together after the lignin had been completely removed has been emphasized.

1. Introduction.

One means of obtaining evidence on the position of lignin in woody tissues is by following microscopically its removal from thin sections of wood by various standard delignifying treatments. To accomplish this successfully, however, it is necessary to be able to demonstrate visually any residual lignin at the various stages of the treatment employed. Suitable methods for revealing the presence of lignin have been discussed in the first paper of this series (4) and it was considered that the methods developed could be advantageously employed in any delignification experiments. By such means it was thought that some valuable information might be forthcoming on (i) the relative amount of lignin in the cell wall and the so-called "middle lamella" zone†,

* An officer of the Division of Forest Products.

† In the present discussion the so-called middle lamella zone refers to the true intercellular layer plus the primary walls of adjacent cells following the terminology of Kerr and Bailey (7).

(ii) the reliability of staining methods for indicating the presence of lignin, and (iii) the process of maceration, i.e., the separation of fibres from each other, which is of tremendous importance in all pulping. Other workers (1, 2, 8) have studied the effects of delignification on wood structure but, in general, have employed rather drastic delignification procedures, and the separation of the individual cells has been rapid. It was decided, therefore, to follow the delignification procedures adopted by Kerr and Bailey (7) and by Van Beckum and Ritter (9) in which lignin only is removed, and to make each stage of the treatment a mild one in order to retard the removal of lignin sufficiently for a series of observations.

2. Material.

For the experiments planned, cross-sections 18μ in thickness were cut from small blocks of wood which had had no other treatment than boiling in water for softening. Numerous such sections were obtained from the species listed below and stored in 10 per cent. alcohol.

Species Used in Present Investigation.

1. Representing class with thick cell wall which had given a distinct cell wall pattern when treated with 72 per cent. H_2SO_4.
   - *Ackama muelleri* Bentham.
   - *Eucalyptus marginata* Sm.
   - *Sideroxylon australe* Benth. and Hook.
   - *Tetramerista glabra* Miq.
2. Representing class with thin cell wall and no definite lignin pattern when treated with 72 per cent. H_2SO_4.
   - *Acacia penninervis* Sieb.
   - *Euroschinus falcatus* Hook.
3. Representing Australian pulpwoods.
   - *Eucalyptus regnans* F. v. M. (mountain ash).
   - *Eucalyptus obliqua* L'Herit. (messmate stringybark).
4. Representing Northern Hemisphere pulpwoods.
   - *Picea* sp. (spruce).
   - *Tsuga* sp. (hemlock).

(Both normal and compression wood examined.)

NOTE.—Of the above species, *Tetramerista glabra, Acacia penninervis, E. regnans,* and *E. obliqua* were utilized for the majority of the experiments.

3. Experimental Procedure.

For delignification the sections were treated with chlorine and the chlorinated lignin removed by an alkaline solvent. In each case chlorination was accomplished in small beakers using chlorine water (strength approximately 6 grams per litre), and at the end of the prescribed time—two minutes only in the majority of the experiments—the chlorine water was removed rapidly and excess chlorine rendered inactive by the addition of alcohol. The sections were then treated for 30 minutes at 20° to 25° C. with the predetermined alkaline solvent for

the chlor-lignin, after which the sections were filtered, washed, treated with very dilute acetic acid (1 per cent. solution in either water or alcohol, depending on the condition of the experiment), and finally washed till neutral to litmus. Washings were either aqueous or alcoholic depending on the treatment. At the end of each such stage of the process, sufficient of the sections were set aside for further experiments, while the remainder were subjected to a further cycle of the operations. Treatments were continued until the sections showed no colour on the addition of the alkali after chlorination or until they became disintegrated or too fragile to handle. The following treatments were employed:—

A. Chlorine water followed by 2 per cent. sodium sulphite (standard as in preparation of cellulose but at 20° to 25°C.).

B. Chlorine water followed by 0.08 per cent. sodium hydroxide.

C. Chlorine water followed by 0.1 per cent. alcoholic sodium hydroxide using alcohol for washing between stages of treatment.

Note.—Treatments B and C were utilized in experiments carried out in the Section of Wood Chemistry of the Division of Forest Products.

D. Similar to C, but with water washes between stages.

E. Chlorine water followed by an alcoholic solution of ethanolamine prepared by adding 3 ml. of pure monoethanolamine to 97 ml. of 84 per cent. alcohol, using alcohol washes between stages (cf. Van Beckum and Ritter, 9).

F. Chlorine water followed by an alcoholic solution of ammonium hydroxide prepared by adding 10 ml. of ammonium hydroxide (Sp. G. .880) to 90 ml. of 84 per cent. alcohol and using alcohol washes between stages. (Compare method of Kerr and Bailey, 7.)

G. Similar to F, but with water washes.

H. Similar to F, but reducing the ammonium hydroxide to 3 ml. and adding this to 97 ml. of 84 per cent. alcohol.

K. Similar to H, but with water washes.

L. Chlorine water followed by an aqueous solution of ammonium hydroxide prepared by adding 10 ml. of ammonium hydroxide (Sp. G. .880) to 90 ml. water.

Certain of the sections removed at the completion of each stage in the delignification process employed were stained with safranin and light green according to the following procedure:—

The sections were stained for one and a half minutes in a 1 per cent. aqueous solution of safranin heating over a small frame. At the end of the period they were washed with 50 per cent. alcohol and then stained in cold light green (1 per cent. in 90 per cent. alcohol) for one and a half minutes. They were further washed in 70 per cent. alcohol, then 90 per cent. alcohol, and finally in absolute alcohol before cleaning and mounting in the usual way. All the sections taken at the various stages of any particular treatment were stained at the same

time so that a direct comparison was possible. In this way the exact stage at which there was any alteration in the colour of the secondary wall and of the middle lamella zone was recorded.

Sections removed at each stage of each treatment were also examined microscopically after the addition of 72 per cent. sulphuric acid (with and without iodine pretreatment) according to the methods previously developed (4).

The results of all the experiments carried out have been tabulated according to the delignification treatment employed (see Table 1).

TABLE 1.—TABLE OF RESULTS.

Treatment.	Species.	Number of Stages for— Maceration.	End of Maüle Colour Reaction.*	Disappearance of Lignin.	Change of Colour of Middle Lamella Zone from Red to Green.
A	*Acacia penninervis*	3	3+	3+	2
	E. regnans	3–4	3+	3	2
B	*Tetramerista glabra*	3	8	3+	3+
	E. regnans	3	3+	2	2
	E. obliqua	3	3+	3+	3+
	Picea sp. (spruce)	3	3+	3+	..
C	*Tetramerista glabra*	8	8	7	6+
	E. regnans	6	6+	4	3–4
D	*Acacia penninervis*	3	3+	..	2
	E. regnans	2	2+	..	..
	Tetramerista glabra	4	4+	4+	4+
E	*Acacia penninervis*	†	8	..	..
	Tetramerista glabra	†	7	3	3
	E. regnans	†	6	2	2
	E. obliqua	†	7	2	2+
	Picea sp. (spruce)	†	8	8+	4
	Tsuga sp. (hemlock)	†	9	9+	4
F	*Acacia penninervis*	†	4+	..	3
	Tetramerista glabra	6	6	2	3
G	*Acacia penninervis*	†	4	4	..
	Ackama muelleri	†	3	3	..
	Acer saccharum	†	5	5	4
	Euroschinus falcatus	†	3	3	2
	E. marginata	5	5	..	5
	Sideroxylon australe	†	5+	4	4
	Tetramerista glabra	4	4	4	3
H	*Acacia penninervis*	†	8	4	3
	E. regnans	†	7	3	3
	E. obliqua	†	7	3	4
	Tetramerista glabra	†	8	3	5
	Picea sp. (spruce)	†	10	10+	..
	Tsuga sp. (hemlock)	†	10	..	..
K	*Acacia penninervis*	†	7	4	..
	E. regnans	5	4	3	3+
	E. obliqua	6	4+	3	4+
L	*Acacia penninervis*	3	3	2	2
	E. regnans	3	3	2	2+

* According to Barlow (6) the Maule colour test is given when a wood is treated with chlorine and then immersed in an aqueous solution of a weak base or basic salt. This is the test referred to in the above table with the exception that both alcholic and aqueous solutions of a weak base were used.

† Sections had not macerated even after all lignin had been removed.

4. Experimental Results.

(a) *Results of Microscopic Examinations for Lignin.*

(i) *Using 72 per cent. sulphuric acid without pretreatment.*—After the first stage of any of the processes no cell wall lignin could be detected except in the case of *Tetramerista glabra* using treatments C and D which apparently did not remove lignin very rapidly. The lignin of the middle lamella zone was apparent in the earlier stages of all the treatments and the speed of its removal depended on the treatment employed. For example in those treatments using alcoholic solutions of ethanolamine or ammonium hydroxide (E, F, and K) it had been removed from the sections of the hardwood species (pored woods) after the first three stages, although the sections had not macerated even after three or more further stages of the treatment. In the case of spruce, however, some residue apparently lignin was observed even after eight to ten stages of the treatment. In this instance there was no consistent parallel between the observed residue and the results of staining (see below).

(ii) *Using 72 per cent. sulphuric acid after iodine pretreatment.*—In the majority of cases no cell wall lignin pattern was observed even after the earliest stages of the delignifying process, but in the case of *Tetramerista glabra* a cell wall pattern was detected up to the end of the second stage. With conditions which apparently retard the solution of the cellulose and the swelling of the secondary wall, it was possible to obtain a clearer picture of the results of delignification in the middle lamella zone. This appeared to be slowly reduced in thickness between cells, but that portion of it in the corners where it always appears thickest remained intact over a much longer period. With treatments E, F, G, H, and K, a splitting in the middle lamella zone was detected on examination of the lignin residues from the latewood of spruce and mountain ash. It appeared as if some portion of the intercellular material itself had been removed by the acid treatment.

As lignin was removed from the secondary wall a definite blue colour (from the iodine and delignified cellulose) developed. This occurred in the earliest stages of delignification and the development of the blue coincided with the change in colour in the staining of the secondary wall (see staining results below). In the case of *Acacia penninervis* and *Euroschinus falcatus* species which have numerous gelatinous fibres, the secondary wall is stained blue even in untreated sections indicating the unlignified state of the gelatinous layer. The middle lamella zone stains reddish brown to brown with the iodine in the early stages of delignification, but the colour is reduced to a pale yellow in the later stages just prior to complete delignification. This portion of the wood never gives the blue colouration of the secondary wall.

(b) *Results of Staining Experiments.*

In the previous paper of this series it was suggested that there appeared to be a high degree of correlation between staining indications of cell wall lignification and the presence of a cell wall lignin pattern. On the basis of these results the application of the double staining procedure to sections removed at the various stages of the delignification

process was a logical step. By this it was found that as delignification proceeded the colour of those portions of the original untreated sections which had stained red gradually changed to green, and in the final stages when delignification was complete those sections which had not macerated were stained green throughout. Further it was observed that the colour change in the middle lamella zone was not coincident with that in the secondary wall, which fact might indicate the slower removal of lignin from that region.

If the red stain of the safranin is accepted as being indicative of lignification, then several interesting conclusions may be drawn on the results of the experiments carried out. With treatments B, C, and D, the colour change from red to green was never complete before the sections disintegrated, indicating that the fibres separated before delignification was complete. The removal of the chlorinated lignin was accomplished quickly by the alcoholic solution of ethanolamine (treatment E), the colour change from red to green being observed one to two stages earlier than when the alcoholic solution of ammonium hydroxide (treatment H) was used. With treatments E, F, and H the staining result indicated complete removal of the lignin (from all woods except the conifers) some time before this had been considered possible on the basis of the Maüle reaction. This was supported by visual evidence when sections were treated with 72 per cent. sulphuric acid. As will be seen from Table 1 there was general agreement between staining results and the observed disappearance of lignin.

However it is well to stress that results of the staining experiments should be treated cautiously even though they are supported by visual evidence. Experiments showed that the thickness of the section used had some influence on the staining of the cell wall. For example, the red colour produced in the cell wall of *Tetramerista glabra* was greatly reduced in intensity when sections of less than 15μ thickness were employed, and with 5μ sections some green colour in the cell wall was detected. Certain mild pretreatments also affect the staining reactions. Clarke (3) found that boiling sections for 4 to 24 hours in water apparently removed lignin as evidenced by the greater tendency to react with the so-called cellulose stain (fast green) in a double staining procedure employing safranin and fast green. Experiments in this laboratory in which sections of various species were boiled for one to three hours in either 0.08 per cent. sodium hydroxide or 1.3 per cent. sulphuric acid prior to staining showed that the staining reactions were considerably influenced by such pretreatments. Sections 15μ in thickness cut from *Doryphora sassafras* and *Tetramerista glabra* when subjected to the pretreatment did not give the normal red stain in the cell wall obtained with untreated sections. When thinner sections (5μ) were used, even the middle lamella zone failed to stain red in the staining procedure. The boiling in water or in the other two reagents does not remove lignin since its presence could be effectively demonstrated in both cell wall and middle lamella zone even after such treatments. It is possible that the stain reacts with a lignin complex which may be slowly hydrolysed and this action is apparently facilitated in the thinnest sections. In the delignification procedures on the other hand the lignin itself is slowly removed and apparently the staining is influenced by this removal. Whatever the mechanics or chemistry of

staining, the fact remains that when sections of standard thickness are employed gradual delignification is accompanied by a change in the staining reaction. The colour of the middle lamella zone and the secondary wall, where "lignified," changes from the red supposedly indicative of lignified tissue to the green of the so-called cellulose stain.

5. Discussion.

Since one of the primary objects of the present investigation was to examine the effects of delignification on wood structure, a recent publication by Bixler (2) is of interest. On the basis of results obtained during the sulphite, soda, and sulphate digestion of spruce 20μ sections, he concluded among other things that the outer portion of the secondary wall is mostly lignin and that when such lignin is removed in the digestion processes there is a gap between the so-called "cambial walls" and the secondary wall (the cambial walls are supposedly the primary walls of Kerr and Bailey which occur between the intercellular layer and the outer layer of the secondary wall). In none of the results obtained in the present investigation was there evidence of such a concentration of lignin or of such a breaking away of the secondary wall layers. The species used included spruce, but none of the delignification treatments called for high temperatures and pressures. It would seem that Bixler's own suggestion that his treatments have gelatinized the secondary wall of the cells and so caused the effect noticed is a much more plausible explanation.

The results of the investigations indicate clearly that the lignin is mainly concentrated in the middle lamella zone (intercellular layer + primary walls), although in certain species, mainly those with thick cell walls, there is a small quantity of cell wall lignin arranged in a definite pattern in the secondary wall. The lignin complex of softwoods (non-pored woods) is somewhat different from that of hardwoods (pored woods), and the actual amount of lignin present in a given dry weight of woods of the former class is generally greater. In addition, a higher percentage of lignin has been recorded for compression wood in comparison with normal wood of the same species (5). It was apparent in the various experiments that under the conditions of delignification employed there was some difficulty in removing all the lignin from the non-pored woods treated. This was in direct contrast to results with the pored woods from which the lignin was apparently removed very rapidly (see Table 1). Those pored woods with definite lignin pattern in the cell wall lost this quickly in delignification but probably there is no differential removal of such lignin in comparison to that of the middle lamella zone. It is only because the latter is present in much larger quantities that it can be detected at later stages.

Allowing for variation between different sections prepared from the one block of wood and the vagaries of the staining technique, it is remarkable that such a high correlation could be obtained between the "lignification" actually observed and that indicated by the stain. Thus there is some definite justification for the claim of the botanists that lignified tissue can be detected by a double staining technique employing safranin and light green and that non-lignified tissues (cellulose) stain green under similar treatment. However, it must be remembered in

interpreting results of staining, first that it is essential for the sections to be of standard thickness, and secondly that staining will give variable results if the sections have been pretreated in any manner.

The colour test generally referred to as the Maüle reaction, which supposedly indicates the presence of lignin by the definite colour obtained after chlorination and treatment with an alkaline solution, may be due to some substance other than lignin. When lignin could no longer be detected by treatment with 72 per cent. sulphuric acid or by staining, the colour reaction was still observed. It is possible that whatever is responsible for the Maüle colour reaction is removed from the wood during delignification more slowly than the lignin, the greater part of which (say 95 to 97 per cent.) is lost during the first two stages of most of the delignification processes used.

The work of Kerr and Bailey was confirmed in so far as it related to the preparation of delignified sections without maceration. It was found, however, that chlorination must be followed by treatment with alcoholic solutions of ammonia or ethanolamine using alcoholic washes between stages to achieve this result. In many instances where water washes or aqueous ammonia were employed, maceration did occur. In addition to employing Kerr and Bailey's alcoholic ammonia as the solvent for chlorinated lignin, a cold 3 per cent. alcoholic solution of ethanolamine was used. The latter treatment is a variation of that developed by Van Beckum and Ritter, who boiled sawdust with a 3 per cent. solution of ethanolamine in 95 per cent. alcohol. In both cases delignified sections were obtained; these retained their original size and shape and were not too fragile to handle. Such sections must be considered as holocellulose but the individual cells must still be held in position by some cementing material other than lignin. Kerr and Bailey state that this material can be removed by standard solvents for polyuronides (pectin solvents), but such a treatment must involve some loss of carbohydrate material. It is therefore evident that the preparation of true "holocellulose" fibres may be impossible, since to free the fibres some loss of carbohydrates must be expected.

According to the discussions of Kerr and Bailey, the removal of lignin alone will leave the intercellular layer and the cambial walls in the same condition as they were in the cambium before differentiation occurred. Reactions are apparently similar if either freshly-cut sections of the cambium or delignified sections of wood are used. It is evident that from the pulping view-point further knowledge of the exact nature of these layers is essential since they must be removed together with the lignin to cause the fibres to separate. The results of investigations carried out on the properties and behaviour of the intercellular layer of several Australian and other species will be discussed in the next paper of this series.

6. Acknowledgments.

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Soil Survey, Erosion Survey, and Land Use Work in America.

*By J. K. Taylor, B.A., M.Sc.**

For forty years the United States of America has been engaged in soil survey work. It is proceeding vigorously now through Federal and State bodies throughout the country. Some States are well advanced and have a complete soil picture of their whole territory in varying degrees of detail; many are still only partially surveyed, but the policy of more and better surveying is followed everywhere. At the present time three groups are operating—(*a*) the Federal Soil Survey Division of the Bureau of Plant Industry; (*b*) the Soil Conservation Service, with headquarters in Washington, D.C.; and (*c*) the 48 State Colleges of Agriculture. By arrangement, surveys are conducted in most States with a team of one Federal and one or more State surveyors, while where the erosion problem is severe the Soil Conservation Service may undertake the whole work.

There are some differences in concept of what a soil survey should do, and more fundamentally as to precision of mapping and soil classification. Almost without exception, the tendency in the last ten years has been towards greater detail and precision, closer definition of soil type characteristics, and clearer realization of the relationship between types based, for example, on topography and internal drainage. Divergence of opinion exists as to groupings of soils, scale of mapping, and whether slope and land use should be included as an accessory feature, but, more than ever before, the soil survey is becoming the necessary foundation on which all experimental, investigational, and advisory work in agriculture is based.

There are three main groups of soil surveys:—(1) detailed surveys such as those sponsored by the Soil Survey Division of the United States Department of Agriculture; (2) erosion surveys as developed by the Soil Conservation Service; and (3) reconnaissance surveys for rapid evaluation of large areas.

1. Detailed Soil Surveys.

In their present form these have moved a long way from the early models. The long accepted practice of mapping with a plane table on a scale of 1 mile to 1 inch has given way to mapping at 20 chains to 1 inch on aerial photographs, with some use of 40 chains to 1 inch topographic maps in the absence of photographs. The detail sought for varies from State to State, largely on the judgment of the individual in charge of State work. For example, Indiana attempts complex mapping in great detail in an endeavour to map out a very complicated drainage pattern. California, on the other hand, leans to a broader

* An officer of the Council's Division of Soils, who recently visited the United States of America.

definition of types and less detail in mapping. In the north-eastern States (typified by Indiana) a system of soil correlation has been worked out based on the "catena" principle of G. Milne*. The soils concerned are largely of glacial origin and may be grouped according to mode of formation—e.g., till, outwash terraces. Within each group several types may be separated on the basis of internal drainage of the soil profile, ranging from excessively drained to poorly drained; it is this sequence of types that forms the American catena. Naturally, it is associated with topography and micro-relief. The principle is sound though not applicable at large—for example, over semi-arid and dry lands generally.

It is a matter of opinion whether a soil map gains or loses in value by including great detail, whether a multitude of soil boundaries in highly diversified country is more readily understood by the average inquirer than a simplified picture showing a "complex of types" without differentiation. The complex covers a gradient of soil types, whereas the individual mapping enforces an arbitrary separation very difficult to keep consistent. It raises the question of what a soil type is, what it was made for, and who is going to use it. The soil type is a classification of soils for utilitarian purposes, for the benefit of the farmer and his advisor. No soil type can be tied down to strict definition like a plant, and mapping soil types becomes an art combining a knowledge of their character with judgment of permissible latitude in detail. Almost universally, the accepted basis for separation of soil types is (not in order of importance)—(1) geological origin of parent material; (2) geomorphology and topography; (3) drainage conditions; (4) vegetation association; and (5) profile morphology. With these points few surveyors would disagree, though some may argue on the relative importance of each.

As far as routine surveying practice is concerned, Australian workers have developed a technique along much the same lines as the American and as effectively, judged by the recent surveys.

2. Erosion Surveys.

Since the inception of soil erosion investigations in 1936, attempts have been made to show the state of erosion according to a specific mapping procedure. The most recent guide to mapping practice is contained in a publication of the Soil Conservation Service in 1939—Soil Conservation Survey Handbook (U.S.D.A. Misc. Pub. 352, 1939). Great detail has been insisted on in accumulating field data which covers four sets of information:—(1) the erosion landscape at the time of mapping; (2) topography; (3) nature and extent of ground cover; and (4) proper use of the land in five classes. The published map shows these five classes in colour dividing the soils into those (1) suitable for general cultivation; (2) suitable for cultivation with simple control measures; (3) suitable for cultivation with complex measures; (4) not suitable for continuous cultivation; and (5) not suitable for cultivation. These groups are decided on for each area and the same soil may figure in several groups depending on slope and present degree of erosion and there is no necessarily fixed grouping for a normal soil between districts.

* G. Milne—*Soil Research* 4: 183, 1935. The American definition of "catena" is somewhat different to Milne's original one.

The topography is shown by slope mapping into five classes, the limits of each being fixed for each survey for soils of certain characters. The slope range within classes may vary from area to area even within one State. The setting up of the legend for mapping purposes is all-important, and there is no guaranteed uniformity of interpretation of classes or symbols.

Erosion is mapped as it appears at the time of survey, and an endeavour is made to assess the amount of surface soil (and subsoil) lost. One set of symbols denotes water erosion, another wind erosion, a third surface accumulation, a fourth gully erosion, adding further a set for stabilized conditions following active erosion. There are in brief many hundred possible groupings to denote a slope-erosion complex. The mapping is of conditions as they exist at the time of survey. It would seem to be much more important to have a generalized assessment of damage, the trend of the erosion process and the erodibility of the soil types. It is also by no means certain that attempting to show slope changes in detail is superior in the long run to making broader topographic separations.

The advantage of having a detailed system laid down for the surveyor is that, with a large staff, it is easier to keep the work uniform and check on such points as the classifying by the surveyor of his soils into the proper land use groups. It is worth keeping in mind that many areas subject to erosion in Australia are not such that they warrant a great deal of detailed mapping being done, and a modification of the American system may give a sufficiently adequate picture without undue expense. A detailed map of a soil erosion landscape may be altered in a single heavy rain or windstorm.

Although the way of map-making may be criticized, the aim, and in most cases the result, are very worthy of attention. America has set herself to planning a permanent agriculture, and this necessitates a stocktaking of marginal and sub-marginal lands and land in various stages of erosion and degeneration. Here then is a pictorial representation of the nature of the soils, the topography, the state of erosion, the present use of the land, and an indication of safe future use. In the well-known "Dustbowl" country of the south-western States, such surveys have provided the groundwork for a map which divides this area of 140,000 square miles into ten "problem area" groups. For example, one group of soils is rated as suitable for row crops, such as cotton, sorghum, another group for wheat and fodder crops, another as grazing land not to be plowed, and so on. The survey of the soil erosion and land use have enabled such a plan for future development to be made and in course of time achieved by example, advice and conditional loans to the farmer.

3. Reconnaissance Surveys.

Up till recent times, the United States Soil Survey made reconnaissance surveys over large areas, mapping approximately on a scale of 3 miles to 1 inch. The maps were not considered accurate and were meant to yield an inventory of the main soils to be found, with some idea of their relative importance. There are, at the present time, two types of reconnaissance mapping being used, different to the original,

which are of value and interest to Australian workers. J. O. Veatch, of Michigan, recognizes what he calls "land types," meaning aggregation of soils fitting into a certain pattern, related to each other physiographically and derived from the same parent materials. There may be considerable differences between the two or more soils grouped, but they have a definable relationship and appear as a recurring pattern in the landscape*. For example, a plain of more or less uniform soils with numerous depressions in which a wide variety of poorly drained soils and peats may be found could be a "land type." Similarly, a sandy plain with windblown sand rises and eroded areas could be mapped as a unit. All the alluvial soil of the present flood plain of a river irrespective of variability is a "land type," but a separation would be made from second, or earlier, river terraces. Once the relationship and pattern are recognized, the groups can be mapped without separating out component soil types, and if this be well done the map is a true framework for any subsequent detail mapping of individual soils. Veatch has used the system successfully in Northern Michigan, mapping as much as 200 square miles per surveyor per month.

With the same idea, but beginning from a somewhat different basis, J. H. Ellis in Manitoba, Canada, maps soils as "associations," and later, as opportunity and necessity arise, separates out the "associates" in detail. This mapping is on sound pedological lines—it seeks to demonstrate the relationship between soils on common parent material under different conditions of drainage or salinity within a zonal group of soils. Manitoba has, for example, the well-known "black earths" as a main zonal group, but there are associations distinguished according to the development of a "black earth" on a lacustrine clay or on coarser deposits round the old lake basin†.

Reconnaissance mapping of the types described could well be applied to much Australian country of low to moderate value and not worth detailed surveying at present. The relationship between types in the "land type" or "association" has to be worked out soundly, and the groupings should have some agricultural significance. An attempt to indicate the pattern of soils which may be expected in one of these groups is made by making a detailed survey of a small number of selected unit areas. The reconnaissance map needs to be as accurate as possible in its boundaries, which should not need material alteration but only subdivision in any later detailed survey.

The second form of these large scale surveys deals with the potential agricultural value of land. Alberta, Canada, has carried out such surveys to sort out the usable soils in the climatically marginal lands in the northern part of the Province‡. For example, the soils were grouped as "grey wooded soils,"—subdivided on the field judgment of the surveyors as first, second, and third class—parkland soils, muskeg (peat bog), and eroded soils. This is sufficient to show the possible use of the country in a broad way, as these types have already been studied under

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* J. O. Veatch—Mich. Ag. Expt. Stn., Spec. Bull. 231, 1933.

† For a full discussion of this system of mapping see J. H. Ellis—*Sci. Agric.* 12: 338, 1932.

‡ See Preliminary Soil Survey of Peace R.—High Prairie—Sturgeon Lake Area, F. A. Wyatt—Res. Council of Alberta, Alta., Canada, Rept. No. 31.

more favorable climatic conditions in experiment plots in settled country. It shows the usefulness of a team of trained soil surveyors in evaluating lands and resources, and in America at large this type of assessment of areas is considered essential before any move in settlement, whether primary or in the way of intensification, is decided.

4. Aerial Photography.

Aerial photographs have long been recognized for their value in topographic work and accurate delineation of surface features During the past five years, they have become of increasing use in America in connexion with mapping crop areas, and since the United States of America has been restricting the planted area of certain crops it has become necessary to have an annually prepared map of all farm lands in the Union. The aerial photograph has supplied the quickest method, affording a base map of farm layout and, at the time of photographing, the land use also. Photographs have been used in soil survey since 1928, when they were pioneered by T. M. Bushnell of Indiana, and in recent years have been universally used.

The photography for the Federal Department of Agriculture and the States is done entirely by contract by private companies who specialize in the work. They use aeroplanes and equipment of special type and are held closely to specifications so that a particularly high standard of workmanship results. The Soil Conservation Service makes field surveys only following photography, and maps all field data directly on the photograph as a permanent record, so that from these the ultimate soil-slope-erosion-land-use map is finally compiled. Approximately 70 per cent. of the agriculturally usable area of the United States of America has been photographed, and the aim is to have a complete picture. The photographs are large prints about 12 inches by 9 inches on a scale of 20 chains to 1 inch. No individual can doubt the usefulness and importance to field workers of the modern aerial photograph. It gives them the topography, a map of surface features in fine detail, the surface drainage, the subdivision of district and farm, the native vegetation, the use of the land and the condition of the crop--it gives the perfect background on which the field party can fill in details. Aerial photographs have been used in many of the soil surveys in Australia by the Soils Division of the Council for Scientific and Industrial Research since 1930.

5. The Tennessee Valley--An Agricultural Experiment.

As an example of modern highly detailed surveying, the attack on the reclamation and improvement of the farms in the Tennessee Valley is worth quoting. The Tennessee River Valley became important in recent years when the United States of America Government set up an agency to control its development and made it the biggest agricultural experiment in the world. The Valley touches seven States which are co-operating with the Federal Government in the effort at land use planning and improved safe production. The standard of agriculture was low and falling, soil erosion was continuous and severe, and living conditions were marginal. The Federal plan was to change all this by advice, loan money, cheap fertilizers, and better social conditions, and

then, by encouraging development of secondary manufacturing industries in the Valley, provide a solid local market for farm produce. The process has not yet gone very far, but it is active and promising.

The first step was the setting up of a soil survey organization to study the whole matter of soil types, erosion, and land use,. county by county throughout the whole Valley. The mapping is considered the best detailed work accomplished in America. J. W. Moon, in charge of this work, makes his surveys cover much more than merely soils and erosion. From the data compiled during a recently completed survey*, Moon drew eight maps showing (1) soils; (2) slopes; (3) erosion; (4) stoniness; (5) drainage; (6) fertility; (7) rotations; and (8) use adaptation. The last one is the summary of the whole survey—it answers the question of what shall be done with this land. The soils having been classified, the topography outlined, the erosion picture painted, and the productivity of the land rated—all this being known to what use are the soils best adapted? For the use of trained extension officers, these maps afford the complete key to understanding the area. This kind of surveying is comparatively slow and expensive even with the aid of aerial photographs, but where the situation demands it, it is well worth while. The field work is entirely the work of soil surveyors; the assessment and land use determination is arrived at by collaboration of surveyors, agronomists, and the advisory service operating in the particular county and State. The whole investigation for the improvement of farm and farmer rests on the fundamental soil survey broadening out into the fields of present and potential uses of the soils.

6. Productivity Rating.

A development which has been growing in practice in recent years has been the rating of soils according to standards determined by observation and experiment. There have been several attempts at mathematical evaluation of soils such as by Kellogg and Ableiter† and R. E. Storie‡, but neither has gained wide recognition for reasons which cannot be discussed in this article. Modifications have been made of both by allotting points for one character or another and treating the data on a kind of score card system. The usual practice in rating the soils for productivity is to consider them in relation to the best soil in the State, or preferably, the physiographic unit in which the survey has been made, for the particular crop concerned. The same soil would possibly rate very differently for different crops as, for example, an excellent tobacco soil might be mediocre for cotton although climatically both crops were suited. In an assessment of soils in the Willamette Valley, Oregon§, one soil, named Olympic loam, was rated as follows:—(ten representing highest productivity) *Olympic loam*—wheat 7, oats 9, potatoes 8, clover 4, apples 8, prunes 6, strawberries 8, pasture 4.

This kind of rating is the result of close study of crop records, experiments, and field observation. In the Willamette Valley mentioned, 56 soils have been rated in terms of twelve crops each. With every survey

* Jefferson County, Tennessee; to be published as a bulletin by U.S.D.A. Soil Survey Division, Bur. of Plant Industry.

† C. E. Kellogg and J. K. Ableiter—U.S.D.A., Tech. Bull. 469, 1935.

‡ R. E. Storie—Ag. Expt. Stn., Univ. of Calif., Bull. 556, 1937.

§ W. L. Powers et al.—Oregon State College, Ag. Expt. Stn., Bull. 365, 1939.

and every county, an attempt is made to give a productivity rating to the various types of soils, and naturally if erosion or misuse has damaged the soils the actual assessment may be very different from the rating of the normal soil type.

7. Land Use Studies.

Spurred on by the action of the Federal Government in initiating the New Deal policy for agriculture, all institutions seem to have placed land use studies in the forefront of their extension work. It is by far the most interesting study in America at the present time, and one which Australia needs to watch for profitable results.

The idea runs through all agricultural thought in America that present conditions are far from satisfactory and must be improved permanently. The slogan is "plan for a permanent agriculture" and first of all go out and make a soil survey combined with field observations on all factors affecting production, such as erosion, soil degeneration, unsuitable soils for specific crops, topography and slope, and drainage. This, supported by the sound study of climatic conditions, forms the basis of all planning, particularly in problem areas of marginal lands or in regions with degenerate soils.

The Population of a Mound Colony of *Coptotermes lacteus* (Frogg.).

By F. J. Gay, B.Sc., D.I.C., and T. Greaves.**

Summary.

Coptotermes lacteus is a common mound-building termite in south-eastern Australia. A brief description of the mound structure is given together with an analysis of the populations of two mounds. The estimated populations were just over 600,000, and 1,100,000 respectively, with a caste composition of 86 to 92 per cent. workers, 6 to 7.5 per cent. reproductive nymphs, and 2 to 3 per cent. soldiers.

These results are compared with data previously obtained for *Eutermes exitiosus*, another common mound-building termite in this region.

1. Introduction.

Two species of mound-building termites, *Eutermes exitiosus* Hill and *Coptotermes lacteus* (Frogg.), have been used for timber and preservative tests and in investigations into control methods at Canberra. Several fundamental studies have already been carried out on the former species, among which was an analysis of the population of a number of mature mound colonies (Holdaway, Gay, and Greaves, 1935)†. More recently, attention has been concentrated chiefly on *C. lacteus*, since it is a representative of the genus responsible for most of the damage done by termites in Australia. An estimate of the population of a flourishing mature colony of this species was required for various reasons, e.g., as a guide in the application of results of laboratory experiments on control by dust poisons.

The only estimate that it was possible to obtain was that of the number of termites present in the mound. This falls short of the total population of the colony by the number foraging away from the nest at the time. By carrying out the population analysis in the winter, the maximum concentration of termites within the mound was obtained. While our observations indicate that in cold weather the foraging activities of *E. exitiosus* virtually cease, *C. lacteus* is much more tolerant of low temperatures, and considerable numbers may be found foraging away from the mound on the coldest days of the year. Thus, even in winter, the population of a mound of this species represents a part only of the colony, although it is reasonable to assume that it does not fall far short of the total population.

2. Size and Structure of Mounds of *C. lacteus*.

Mounds of *C. lacteus* are conical (see Plate 1, Fig. 1) or dome-shaped structures and vary in size according to the habitat. In forest country in the Australian Capital Territory, the average mound has a basal diameter of about 6 feet and a height of 4 feet. In open grazing country the corresponding average measurements would be 4 ft. 6 in. and 2 ft. 6 in. The mounds are characterized by an outer wall of clay containing very few galleries and varying in thickness from 6 inches to as much as

* An officer of the Division of Economic Entomology.

† Holdaway, F. G., Gay, F. J., and Greaves, T. (1935).—This *Journal* 8: 42-46.

24 inches. The space within the protecting clay wall is occupied by a core of tough woody material containing an intricate network of galleries. The structure of this woody core varies in the different regions of the mound. The greater part of it is sponge-like in appearance, here and there becoming more lumpy and containing fewer galleries. In the so-called nursery (that region of the mound in which the eggs and young stages are found) the galleries form a system of concentrically arranged flat chambers, and the separating walls are thin and brittle. Above the nursery, which is usually at or near ground level in the centre of the mound, the woody material tends to form a mass of interlocking irregular lumps, so lightly fused together that they can be separated without difficulty.

Samples of the woody core from two regions of a mound, where the structure was respectively sponge-like and lumpy, were subjected to ignition tests. The results showed that the percentage of organic matter did not differ significantly, being 91.9 and 93.1, and indicated that the insects employed the same material in the construction of the different regions of the mound core, this material, almost certainly, being semi-digested wood.

When exposed (see Plate 1, Fig. 2), the woody core of a *Coptotermes* mound is seen to be pyriform in shape, spreading out at and below ground level. In some mounds this lateral spread is sharply accentuated to form a distinct verandah-like extension beneath the soil surface. The convex base of the woody core is but loosely attached to the surrounding soil, and when prized up reveals the seat of the mound as a smooth saucer-like depression in the ground, 8 feet or more in diameter. Some idea of the mass of material in a mound of *C. lacteus* may be gained from the fact that in the second mound used in the present study, the weight of woody core material brought into the laboratory, for the separation of termites, was 6 cwt. Approximately 2 cwt. of similar material was not collected because it contained scarcely any termites. In addition to this woody core, the solid clay outer wall weighed several hundredweights, so that the total mass of the mound structure was well in excess of half a ton.

The distribution of termites in the mounds examined has been very constant in that the portion of the mound above the nursery rarely appears to contain more than a few hundred termites. The bulk of the population is present either in the nursery, the sub-surface "verandah," or the basal spongy material.

3. The Analysis of the Populations of two *C. lacteus* Mounds.

The two mounds used in this study were similar in size but were obtained in different localities. Mound 1, which was studied on 18th July, was located in forest country in the Brindabella Range, Australian Capital Territory. Its horizontal dimensions at ground level were 6 ft. 3 in. by 5 ft. 6 in., and its height was 3 ft. 4 in. Mound 2, studied on the 27th July, was located in open grazing country near Gundaroo, New South Wales. This mound was somewhat larger than the normal size in open country, its horizontal dimensions at ground level being 6 feet by 5 ft. 6 in., and height 3 ft. 2 in.

The first population study was made in the field, and consisted of stripping off the outer clay wall, breaking up the woody core and jarring

and sieving the termites from this material. Unfortunately this study was interrupted by rain and an imperfect separation was obtained. It was estimated that the recovery of termites from this mound material was only 50 per cent. of the total number present.

In the second study, the mound was brought into the laboratory and the termites removed by the same methods. Here, with the benefit of previous experience and more time, a much better separation resulted, and it was estimated that 80 per cent. of the termites present in the mound material were recovered.

We realize that estimates of percentage recovery, such as those just given, are open to criticism, but it is hard to see how they could be avoided, since it is impossible, in practice, to effect a complete separation of the termites from the mound material. Although the estimates given were based on mere visual impression, this was backed by the experience gained in the separation and counting of termites from scores of mounds that have been brought into the laboratory for testing purposes. The figures, 50 and 80 per cent., were arrived at independently by both of us, and were if anything high: that is to say the actual numbers of termites present in the mounds were probably greater than the estimated populations given below.

The method of estimating the mound populations and the percentages of the constituent castes was essentially the same as that employed in the Eutermes study referred to above. A series of small weighed samples were taken at random from the massed termites, which had been separated into trays, and the numbers of each caste in the samples counted. From the total weight of the termites separated from the mound, and the averaged results of the sample counts, the numbers of each caste in the separated termites were calculated. Part of the population of each colony was used for experimental work, and had to be analysed separately, thus complicating the procedure somewhat. In the case of one of the mounds studied it was unfortunately impossible to deal with the main bulk of the separated termites in the live state. However, the sampling and weighing technique applied to spirit-stored specimens, which it was necessary to adopt, is known to be reliable. It has been employed in certain of our laboratory experiments in which accurate estimates of numbers are required, and it has been found to give an error of only about 1 per cent., provided that a standard method of drying is adopted.

Mound 1.

328 g. of termites were used for laboratory experimental work. Four 5 g. samples of these termites had an average composition per g. of 219 workers, 13 nymphs, and 3 soldiers. Totals of the castes in the bulk of 328 g. were in round numbers, 72,000 workers, 4,300 nymphs, and 1,000 soldiers.

The remainder of the termites separated were stored in alcohol for some time, then air-dried and sampled. 940 g. of termites were treated in this way, and six samples of 5 to 7 g. had an average composition per gramme of 224 workers, 15 nymphs, and 5 soldiers. Thus, the total numbers of each caste amongst the alcohol-stored termites were 211,000 workers, 14,100 nymphs, and 4,500 soldiers.

The total numbers of termites separated from the mound were, therefore :—

283,000	workers	92.2 per cent.
18,400	nymphs	6.0 per cent.
5,500	soldiers	1.8 per cent.
306,900	all castes.	

The estimated separation of 50 per cent. gives an approximate total of 614,000 termites present in the mound material.

Mound 2.

Of the termites separated from this mound, 715 g. were used for laboratory experimental work. As only soldiers and workers are required for this work, juvenile forms were excluded as much as possible and all reproductive nymphs excluded. Ten 2 g. samples of these termites had an average composition per gramme of 254 workers, 6.4 soldiers, and 0.5 juveniles, so that the total numbers of each in the bulk lot were 181,500 workers, 4,500 soldiers, and 300 juveniles. The removal of nymphs from this bulk of termites was, of necessity, accompanied by the removal of soldiers and workers and during this operation 13,500 nymphs, 11,500 workers, and 300 soldiers were removed.

The remainder of the termites from this mound weighed 2,735 g. Ten 2 g. samples of these termites showed an average composition per gramme of 208.4 workers, 19.5 nymphs, 7.6 soldiers, and 11.3 juvenile forms. Thus, of the 2,735 g. the total numbers of each caste were 570,000 workers, 53,200 nymphs, 21,000 soldiers, and 30,700 juveniles.

The total numbers of termites separated from this mound were therefore :—

763,000	workers	86.1 per cent.
66,700	nymphs	7.5 per cent.
25,800	soldiers	2.9 per cent.
31,000	juveniles	3.5 per cent.
886,500	all castes.	

The estimated separation of 80 per cent. gives an approximate total of 1,108,000 termites present in the mound.

4. Discussion.

It is evident from the above figures that although the two mounds were similar in size, there was quite an appreciable difference in the populations associated with the two mounds. In this connexion it is interesting to note that in Mound 1, no queen was found, nor were there any eggs or first instar forms present. This suggests that this colony may have been temporarily at least, below its normal strength. On the other hand, the queen was collected from Mound 2, and large numbers of eggs were present, indicating that the colony was quite normal.

In the study made on *E. exitiosus* (cited above), populations of four mounds ranged from just over 750,000 to over 1,750,000. The latter figure was obtained after the coldest snap of the period of the study and is probably the most accurate indication of the size of the population of mature flourishing colonies of this species.

It will be seen that the mound population of *E. exitiosus* is normally in excess of that of *C. lacteus*. However, as previously mentioned, observations over a number of years have shown that considerable numbers of workers and soldiers of *C. lacteus* occupy foraging galleries during the winter, while a colony of *E. exitiosus* is almost completely confined to the mound during the colder weather, and very few individuals are found in foraging galleries at this time. In this connexion, it should be mentioned that during the separation of termites from Mound 1, termites from this colony were found working in logs in the vicinity of the mound. It would follow from this that the estimates of the populations of mounds of *E. exitiosus* are closer to the total populations of the colonies than are the figures given above for *C. lacteus*, and it is probable that if an absolute count of the populations could be made, the difference between the sizes of populations of mature colonies of the two species would be less than these estimates indicate.

The population studies of *C. lacteus* and *E. exitiosus* indicate that there may be a marked difference in the proportions of the castes in colonies of the two species. In comparison with the figures already given for the *C. lacteus* colonies (workers, 86 to 92 per cent., soldiers, 2 to 3 per cent., nymphs, 6 to 7.5 per cent.), the proportions of the castes in the four *E. exitiosus* colonies studied were:—workers, 83 to 90.5 per cent., soldiers, 7.5 to 12.5 per cent., nymphs, 2 to 7.5 per cent. In order to determine whether this relatively high proportion of soldiers was typical of the latter species, the percentage of soldiers in 46 mound colonies of *E. exitiosus* brought into the laboratory for testing purposes, has been calculated. (The figures given are percentages, not of the total population, but of workers-plus-soldiers: the resulting error, however cannot be great. It should also be mentioned that in separating the termites into trays for use in laboratory colonies, those portions of the mound containing large numbers of juvenile forms are discarded; therefore the separated termites, which usually number between one and two hundred thousand, may not always represent a true sample of the population of the colony.) In 39 of the 46 colonies, the proportion of soldiers was between 4 per cent. and 15 per cent.; six colonies had over 15 per cent. (one even over 20 per cent.); while one colony had as few as 1.5 per cent. soldiers. These figures confirm those obtained in the main population study, and indicate that the proportion of soldiers in *E. exitiosus* colonies is normally much greater than in colonies of *C. lacteus*.

The Subterranean Foraging Galleries of *Coptotermes lacteus* (Frogg.).

By F. N. Ratcliffe, B.A. and T. Greaves.**

Summary.

(1) The subterranean foraging galleries radiating from a mound of *Coptotermes lacteus* (Froggatt) were traced. From fifteen main trunks, branches ramified over an area of approximately 1½ acres, and connected with dead wood lying up to 50 yards from the mound. The method of tracing the galleries, and their characteristics, are described; and the means by which the insects locate food is briefly discussed.

(2) The number of foraging galleries radiating from each of two other mounds of this species, located in a different type of habitat, was found to be over thirty.

(3) The galleries associated with five mounds of *Eutermes exitiosus* Hill, a species with habits similar to those of *C. lacteus*, were investigated. The galleries of the former termite showed a greater tendency to connect with one another and form a reticulating system than did those of *C. lacteus*, in which the main trunks and their branches usually remained separate.

1. Introduction.

Coptotermes lacteus (Froggatt) is one of the two common mound-building, wood-eating termites found in south-eastern Australia, the second being *Eutermes exitiosus* Hill. Mounds of both species occur in forest, savannah woodland, and ring-barked grazing country; and the insects themselves may be found attacking dead trees, stumps, and logs, and also fence posts, poles, and timber structures, some distance from their nests. To reach these food supplies, each colony excavates a number of foraging galleries which radiate outwards from the mound below the surface of the soil. To the best of our knowledge, the system of foraging galleries associated with a populous termite colony has never been investigated in detail.

In addition to providing data on the foraging range of a characteristic sound-wood attacking termite, and, it was hoped, throwing some light on the manner in which the insects located their food, this investigation was undertaken with the object of clearing up a rather puzzling problem that had arisen during the course of the timber testing carried out by the Division of Economic Entomology at Canberra. The resistance to termite attack of different timbers, and of timbers treated with various chemical preservatives, is assessed by burying samples around mounds. It had been found that among those samples installed around mounds of *C. lacteus* the incidence of attack was lower than in the series installed around *E. exitiosus* mounds; and it was suspected that this might be due to a difference in the arrangements of the galleries in the two species. Before the gallery studies described in this paper were completed, the practical problem presented by this difference in the incidence of attack on samples had been solved by a method which need not be discussed here. This was just as well, for it was found that the galleries in the soil immediately surrounding the base of a mound tended to branch and connect with others at different levels, and to form a complex three-dimensional pattern which was exceedingly

* An officer of the Division of Economic Entomology.

difficult to elucidate. At about 8 feet from the centre of a mound (of *C. lacteus*) it was found that the main foraging galleries had become distinct; and attention was therefore concentrated on that part of the gallery system lying outside the 8-ft. circle.

The distribution of the galleries around some mounds of *Eutermes exitiosus* was investigated; and a brief description of the results obtained is included in this paper, as they indicated that there might be differences between the gallery patterns in the two species.

2. The Foraging Gallery System of a *C. lacteus* Colony.

(i) *Site Selected and Preliminary Survey.*

This study was carried out on an area of lightly-timbered grassland on a pastoral property near Braidwood, in the Southern Tablelands district of New South Wales. A large proportion of the trees had been ringbarked or felled many years previously, so that there was abundant food for wood-eating termites in the form of stumps, logs, and fallen branches (see Plate 2, Fig. 1). Mounds of *C. lacteus* were numerous; and another *Coptotermes*, *C. frenchi*, which nests in the butts of living trees or underground, was common in the locality and was feeding on the dead timber lying about.

The mound selected for study was 2 ft. 6 in. high, and had a basal diameter of 4 feet, being of the average size of mounds found in open country, which do not attain the dimensions of those occurring in the forest. It was sufficiently far from any other mounds to render it unlikely that there would be complications due to its territory overlapping that of another colony. The nearest mounds were 200 yards to the N.N.E., 200 yards to the E., and 170 yards to the E.S.E., the last-mentioned being inhabited by a moribund colony. A creek, the bed of which was eroded to a miniature canyon nearly 10 feet deep, ran in a S.E. to W. direction, approaching to within 40 yards of the mound on the S.-S.W. side. The ground was level between the creek and the mound, but sloped gently up from the latter in an easterly direction. (Gallery No. X followed approximately the line of the steepest slope.)

Below the humus-darkened surface layer, the soil was a pale yellowish-grey loam, becoming distinctly sandy at a depth of a foot or less. The subsoil, which was only disclosed at one or two points during the digging, was a brick-red clay. In places on the slope mentioned above, a layer of gravel was encountered above the clay; and here the sandy layer was not well marked. The gravel band varied in thickness, sometimes exceeding 6 inches in depth.

Before starting to trace out the galleries, all logs and stumps within 100 to 180 yards of the mound were examined, and their condition and the presence or absence of termites in them noted. Termites were found working in some of the most distant logs. *C. lacteus* and *C. frenchi* are very similar in their sterile castes; and it was not until specimens collected from the different logs in the surveyed area were examined in the laboratory that the true state of affairs was disclosed. It was then found that the termites from the mound were confining their attention to timber within a radius of 50 to 60 yards. Outside the limits of the activity of the mound colony, the logs were being attacked by *C. frenchi*, until one approached the three other mounds referred to above, when *C. lacteus* was again found working in the dead wood. It

was thus evident that the foraging territory of mound to be investigated was separated from those of other colonies of *C. lacteus* by a zone in which *C. frenchi* was active. The two species were never found together in a log or stump; but *C. frenchi* was found in one or two logs which, though well within the foraging range of the mound colony, had escaped the attention of its inhabitants. (These logs are omitted in the plan of the gallery system of this mound.)

(ii) *The Tracing and Mapping of the Gallery System.*

The main trunks of the foraging galleries were intercepted and located by digging an encircling trench to a depth of 12 to 15 inches at a distance of 8 to 10 feet from the centre of the mound. As it was found that a spade cut obliterated all signs of even the largest galleries, the digging was done with the blade of the spade transverse to the line of the trench, leaving a natural fracture on either side. One observer examined this while the digger carefully broke up the freshly lifted clod of earth in his hands. Sometimes the man examining the sides of the trench was able to anticipate the discovery of a gallery in the clod. Usually, however, the gallery was disclosed by the clod breaking across it. Then, when its depth and direction had been noted, it was possible to locate and to mark the points at which it entered and left the trench.

Each gallery, and its various branches, was then traced outward from the trench. Progress was most sure and rapid when two men worked together. One removed the soil above the gallery with a spade. The other (who directed his partner by indicating the depth of the gallery and the direction it appeared to be taking) cleared the gallery with a knife or trowel, leaving it, when the spade had not dug too deeply, as a ribbon along the base of the extending trench (see Plate 2, Fig. 2). With care and practice it was found possible to manipulate the spade so that the clod broke away across the gallery, retaining the roof and leaving the floor exposed in the trench.

To facilitate the tracing of the galleries, powdered talc dyed with methylene blue was blown into them. Two types of blower were employed. For the larger galleries a rubber motor-horn bulb with a glass or ebonite nozzle worked well enough; but for the smaller galleries a blower with a fine nozzle and a more powerful blast was required. A De Vilbliss No. 36 powder blower was selected, and proved very satisfactory. It was usually necessary to clear the opening of a gallery at the point where it disappeared into the unbroken soil. When this had been done, the powder could be blown to a distance of 2 to 3 feet or more along the larger galleries, and 6 inches or so along the smallest, unless termites were numerous in the galleries.

In preparing the plan of the subterranean gallery system (see Fig. 2*), the positions of the various logs, &c., in which the termites were operating were first determined by their compass bearings (to the nearest degree) and their distances from the centre of the mound. In the case of large logs lying close to the mound the two ends were plotted in this way, obviating the necessity of measuring the logs and the directions in which they lay (as was done in the case of logs lying further from the mound). The galleries were then drawn freehand on the plan, after the more important points along them (i.e., the positions of the branches and the extreme points of the major curves) had been fixed by compass

* See folder facing page 160.

bearing and measuring tape. The plan therefore does not follow faithfully the minute details of the wanderings of the galleries; though an attempt was made to reproduce these as far as possible.

It will be seen from the plan that one or two galleries were not traced right out*. These were either well-used galleries leading to an *obvious* food supply (e.g., the main trunk of No. XIII), or small, presumably exploratory galleries, which could not link up with an established food supply (e.g., branches of No. V). In no instance did we neglect to follow up a gallery which, to judge by its size and direction, could have led to logs further out from the mound than those to which galleries had been traced.

Apart from a number of small unlined galleries that appeared to peter out in the soil, three galleries were lost among the roots of trees. Two of these (branches of Nos. VII and XI) were small and poorly lined. The third, the main trunk of No. XII, was a large and well-lined gallery which seemed likely to serve more than one food mass. It was lost under the bole of a living snow gum; and we failed to pick it up on the far side. It appeared probable that a branch from this gallery might lead to the large log (bearing 58°, 150 feet from mound) in which *C. lacteus* was working. An attempt was made to pick up a gallery near the log, but by this time the soil had dried out and become hard and dusty, and no serving gallery could be discovered.

(iii) *Features of the System and of Individual Galleries.*

Fifteen galleries were intercepted at the trench; and for convenience of reference these were numbered arbitrarily I, II, III....XV, in a counter-clockwise direction round the mound, starting from the largest gallery, which was the first to be traced out. From these main trunks, branches ramified over an area of about 1½ acres, connecting with food masses some of which were over 50 yards from the mound. The aggregate length of the galleries traced was approximately half-a-mile.

The galleries had the "freckled," mosaic-like lining so characteristic of *Coptotermes*, which, in this case, was dark chocolate brown dappled with lighter flecks. This lining, which appears to be formed of dried fecal droplets, was so intimately associated with the surrounding soil that it could not be separated from it. (In contrast, the lining of the *C. frenchi* galleries that were picked up and traced, though similar in appearance, could usually be flaked away from the soil.) As this lining must take some time to be formed, it was not surprising to find no sign of it in those galleries which were in process of construction. It was noticed that the soil immediately surrounding old galleries (known to be such because they served large food masses which had been practically demolished by the termites) often had a yellowish discolouration; and it is suggested that this is due to some substance diffusing slowly from the lining material.

The largest gallery—No. I—had a width of 1–1¼ inches for some distance from the mound. The main trunk of No. XII was over ¾ inch wide, and not much less than ¾ inch at the point where it was

* The work of excavating the galleries shown on the plan, and the following out of one or two *C. frenchi* galleries on the outskirts of the mound territory, occupied fourteen days, with four men working most of the time. The call of other work made it necessary to wind up the investigation at this point.

lost. Nos. XIV and XV were about $\frac{3}{4}$ inch wide before branching. With the exception of No. IV (which faded out within a few feet of the trench, after passing under No. III.), the other main galleries were all less than $\frac{3}{4}$ inch in width, most being about $\frac{1}{2}$ inch wide. The smallest galleries traced were hardly wide enough to allow two termites to pass, being of the order of $\frac{1}{8}$ inch wide. The galleries remained extraordinarily constant in width between branches. In tracing a gallery, when a marked change in its width was noticed, a careful search would be made at once for lateral branches that might have been missed; and on more than one occasion an important branch was discovered in this way. When noted in relation to the distribution of food, the widths of the different galleries and their branches seemed to reflect the volume of traffic which they would be called on to carry.

In the plan, all galleries, down to the smallest branches, are indicated by lines of uniform width. In order to show how the width of a gallery varied with its ramification, the approximate widths of certain "reaches" and branches of gallery No. I are shown on the plan.

However wide the galleries might be, they rarely exceeded $\frac{1}{8}$ inch in height. Thus, the larger galleries were in the nature of horizontal slits in the soil, a fact that made their discovery by the process of breaking the lifted clods relatively simple.

Very few galleries ran at a depth greater than 9 or less than 3 inches below the surface. At the points where they were intercepted by the trench dug around the mound, the main galleries varied in depth from 8 to $2\frac{1}{2}$ inches, the shallowest being those on the southern side, where the clay subsoil approached most closely to the surface. Except in the neighbourhood of scattered food, very few galleries were found in the darker, more crumbly, root-matted surface soil. Gallery No. III, however, was very shallow for some distance out from the encircling trench, in places approaching within an inch of the surface.

The termites apparently evinced a marked dislike for tunnelling in the gravel layer occurring on the east and south-east of the mound. With the exception of No. IX, the galleries ran on top of the gravel layer, varying in depth with the depth of the gravel. Gallery No. IX ran above the gravel, at a depth of 6 to 8 inches, for over 100 feet (to the point marked S on the plan): then it suddenly dived vertically, penetrating the gravel layer and reaching the red clay subsoil. From this point until (by following up an old root) it came to the surface under an old log, it ran at a depth of some 15 to 18 inches, and followed an exceedingly erratic course, which suggested that the tunnellers had been uncertain of their bearings. The extension of this gallery beyond the above-mentioned log ran at a depth of 2 to 3 inches below the surface to serve an old stump surrounded by fallen branches.

When two galleries crossed at different depths, they might or might not be connected; and the presence or absence of a connexion did not depend on the differences in the depths. Thus, gallery No. IX passed under Nos. X and XI without any connexion, although the difference in depths was a couple of inches at most. Only 1 inch of soil separated galleries Nos. XIII and XIV where they crossed; and, it was definitely established that there was no connexion between them. Beyond the food masses served by galleries Nos. VII and VIII, the former crossed

the latter at the point marked SC on the plan. No. VIII was here 5 inches or more below No. VII ; but the two were connected by a wide, vertical, shaft-like gallery.

Beyond the shaft connexion just referred to, gallery No. VIII seemed to come to an end among a number of irregular cavities about a foot below the surface of the soil. Similar cavities, often of considerable size, are found associated with mounds of this termite, usually below the periphery. It is believed that they are formed as a result of the insects mining for the soil—particularly soil with a high clay content—used in the construction of the outer wall. *Coptotermes* have the habit of bringing clayey soil, often in considerable quantities, into the timber which they have hollowed out. Possibly the cavities to which gallery No. VIII led were associated with some such activity.

At various points along the galleries, but particularly in the neighbourhood of food masses, vertical "shafts" occurred. An attempt was made to discover what happened to some of these; but it was only possible to ascertain that they penetrated to a depth of 1 to 2 feet, and reached the clay subsoil. The most plausible explanation of the occurrence of these shafts is that they enable the foraging parties, during the dry season, to tap the moisture held in the lower levels of the soil, and thus to avoid the necessity of returning to the mound when they began to feel the ill effects of a low humidity. The positions of those shafts marked during the excavations are indicated on the plan by the letter S. Unfortunately, some of those discovered were not marked; and many more must have escaped notice.

Examination of the plan will show that when a gallery reached a food mass it more often than not continued beyond it, either as an exploratory tunnel or to connect with other food. The connexion between a gallery and a food mass generally took the form of a steeply-rising branch coming off at a point either directly under or very close to the log. The gallery itself, when travelling further, would maintain its depth, and without faltering in its course pass under the log up to which the "feeding" branch or branches had risen.

(iv) *The Location of Food—Exploratory Galleries.*

The foraging galleries of the colony investigated displayed two characteristics which are obvious in the plan. (1) Although they might lead, with reasonable directness, to the food masses served, the detailed meanderings were such that, during its course, a gallery rarely pointed in the exact direction of the log with which it ultimately connected. This indicates that the termites do not possess a mysterious sense of direction, which some observers might be tempted to attribute to them. (2) Galleries serving distant food masses tended to reach them via other logs lying closer to the mound. This is what might have been expected, since it would simplify the process of food location; and it probably takes place to a greater extent than the plan alone indicates. There must have been, at one time, more food lying close to the mound, which had been eaten out during the early years of the colony's existence. What appeared to be the remains of logs occurred here and there between the mound and the nearest existing food masses. Where these were represented by a definite shell, they are indicated on the plan by broken outlines.

As has already been mentioned, it was hoped that this investigation would throw some light on the termites' method of locating their food supplies and directing their foraging galleries. Although some interesting and possibly significant facts were revealed during the tracing of the galleries, it must be confessed that the ability of the insects to locate logs lying on the surface of the ground some distance from their nest, and to reach them by subterranean tunnelling, remains unexplained.

The possibility that surface scouting plays some part in the discovery of food cannot be dismissed, particularly as it is known that soldiers of *Eutermes exitiosus*, a termite whose habits are very similar to those of *C. lacteus*, sometimes leave the shelter of the nest and galleries at night to wander freely in the open. If there is no surface scouting, one must assume that the whole foraging area—in the case of the colony studied, approximately $1\frac{1}{2}$ acres—is explored by subterranean tunnelling, the galleries which managed to connect with new food presumably being enlarged into, and remaining as, the foraging runways. Even if surface scouting does take place, there remains the problem of how the insects direct a gallery some inches below the surface to reach an object located in this way.

It will be noticed that two of the ultimate branches of gallery No. I extend for a considerable distance beyond the food masses served to end blindly in the soil. These galleries were among the smallest traced; and they were traced right out to what appeared to be the pitface, so to speak, at which the insects were working. An interesting feature of these galleries was the occurrence, at intervals along their length, of vertical shafts which led up to the surface of the soil. These galleries might not have been normal exploratory tunnels, since it is conceivable that their excavation was stimulated by the cutting of the connexion with the central nest. (Five to seven days had intervened between the digging of the trench round the mound and the tracing of these branches.) Nevertheless, it seems reasonable to suppose that even though these particular galleries might have been constructed under an abnormal stimulus, the normal habits of the insects were displayed during their excavation. Thus, it may be concluded that when tunnelling underground the termites always have, one might say, an eye on the surface.

It was not only in the ultimate, presumably exploratory, branches that surface shafts were found. Three or four were disclosed during the tracing of the main trunk of gallery No. I which, just beyond the encircling trench, was packed with termites intercepted on their return to the mound. These shafts, which led up from the roof of the gallery, appeared to be freshly excavated, and sometimes connected with a honeycomb of little galleries among the grass roots. One or two short side branches from the main trunk of this gallery also appeared to turn upward, almost at once, to reach the surface of the soil.

The use which termites make, or might make, of roots perhaps deserves mention. Gallery No. IX seemed to reach the log which it served as a result of a chance contact with an old root. Many of the food masses were felled trees; and it is obvious that the radiating root system would offer an extensive target, so to speak, for an exploratory gallery, which, once it had made contact with any root, would be led to the centre of the system and the neighbourhood of the base of the

fallen trunk. It is not unlikely that several of the galleries reached the larger logs by following up the remains of roots; but an examination of the plan leaves little doubt that the course of some galleries near food must have crossed, rather than followed, the roots of the fallen trees which they served. (When a gallery was found running in the recognizable remains of a root, this has been indicated in the plan.)

3. The Foraging Galleries of Two other *C. lacteus* Mounds.

This investigation was undertaken in order to determine whether the foraging gallery system described above was typical of *C. lacteus* colonies as far as the number and depth of the radiating galleries were concerned. The two mounds selected for study were within a quarter of a mile of one another in the forest-covered Brindabella Range to the west of Canberra. Mound A was on the edge of a large, open, grassed area, and had the dimensions of an open-country mound (height, 2 ft. 4 in., basal diameter, 4 ft. 3 in.). Mound B was in the forest, which was rather open on the northern side, permitting moderate insolation of the mound. In its dimensions it was typical of the large mounds found in densely-timbered country (height, 4 feet, basal diameter, 6 feet). The temperatures of both mounds indicated that they were inhabited by normal vital colonies. An encircling trench was dug at a distance of 8 feet from the centre of each mound; and the information obtained is summarized below.

(i) *Mound A.*

Distribution of Food.—The available food was confined to the S.E.–S.W. sector (the forested side of the mound), and comprised a large standing dead tree 54 feet to the S.E. of the mound, another 120 feet to the S.W., and between the two, three large fallen trees, two stumps, and one standing dead tree.

Distribution of Galleries.—This in no wise reflected the extraordinarily unbalanced distribution of the food, though it should be noted that the three galleries which exceeded 1 inch in width were all on the southern side of the mound. No less than 61 galleries were located on the inside face of the trench; but of these only 36 could be picked up on the other side of the trench. The number, width, and depth of the "straight through" galleries in each of the four sectors were as follows:—*North to east*, eight galleries, depth 6 to 13 inches (average depth 8½ inches); 0.4 to 0.8 inch wide. *East to south*, ten galleries, depth 6 to 14 inches (average 10); 0.4 to 1.2 inches wide. *South to west*, ten galleries, 5 to 14 inches (average 7.5); 0.4 to 1.3 inches wide. *West to north*, eight galleries, 5 to 12 inches (average 7); 0.4 to 0.8 inch wide. In addition to these galleries, the trench disclosed numerous irregular cavities at varying depths in the soil. Most of these were concentrated between the W. and S.W. points, though a few occurred in other parts of the trench.

(ii) *Mound B.*

Distribution of Food.—Dead wood lay on all sides of the mound. Nine food masses lay within 20 yards, and thirteen within 40 yards of the mound, most of them being large fallen or standing dead trees.

Distribution of Galleries.—The number, depth, and width of the foraging galleries intercepted by the trench were as follows:—Total number, 36. *North to east,* seventeen galleries, depth 8 to 18 inches (average $10\frac{1}{2}$); all just under or just over 0.5 inches wide. (A large dead tree, just outside the trench, would account for the numerous small galleries in this sector. Some of the galleries followed the course of, or were actually excavated in, its roots.) *East to south,* eight galleries, depth 5 to 17 inches (average 10); 0.4 to 1.0 inch wide. *South to west,* five galleries, depth 6 to 12 inches (average 9); 0.4 to 1.0 inch wide. *West to north,* six galleries, depth 7 to 18 inches (average 11); 0.3 to 0.8 inch wide. In addition to the foraging galleries, irregular cavities were disclosed by the digging of the trench, just as in Mound A. A few of these cavities had a dappled lining, like that of the galleries, but most were unlined, 12 inches or more below the surface, and either just above, or within, the puggy red clay subsoil. A stony layer occurred in places, and was penetrated by a few galleries. Between the S. and S.E. points this layer extended more deeply than elsewhere, and here the galleries ran beneath it in the red clay.

The data from these two mounds suggest that the colony selected for the complete gallery study previously described had an abnormally small number of foraging galleries radiating from the mound. On the other hand, it is conceivable that the gallery system was typical of a colony in that particular habitat (partly-cleared savannah) and in that stage of its existence when the close-lying food had been used up, and outlying logs were being attacked. When the colony had been concentrating on food lying within, say, the 20-yard circle, there may have been several additional galleries which later fell into disuse and were blocked by the termites. In this connexion, it is worth mentioning that a disused and blocked gallery was discovered between the points where Nos. VIII. and IX. left the trench; and it was possible to trace it for some feet by the discolouration of the soil. If there had been more of these galleries, they would probably have escaped notice, for the one discovered was only picked up by a lucky chance.

The extraordinary multiplicity of galleries around Mound A was certainly abnormal, and can be attributed to the fact that this mound was used during 1935 and 1936 for testing the resistance of timber samples, the installation of which must have disturbed the colony and led to a re-organization of the galleries in the immediate neighbourhood of the mound.

4. The Foraging Galleries of *Eutermes exitiosus*.

The number and depths of the foraging galleries radiating from five mounds of *Eutermes exitiosus* were determined by the method employed in the *C. lacteus* studies. The mounds of the former termite are smaller, and the encircling trench was dug at a distance of 5 feet from the centre of each mound. The mounds investigated were uniform in size, all having a basal diameter of about 3 feet, the heights varying from 10 to 14 inches. The nursery temperatures indicated that all were inhabited by vital colonies.

The mounds studied lay within a relatively small area of cleared grazing land, which had once been open savannah woodland. Within this limited area, however, the soil conditions were found to vary considerably. *E. exitiosus* colonies were abundant, as is indicated by the fact that there were five mounds within 150 yards of the first mound studied (the actual distances being 65, 70, 90, 95, and 130 yards). Although galleries from one of the mounds were traced to food lying over 80 feet from the nest, and almost certainly extended to some logs 20 feet further away, our investigations were not sufficient to determine the foraging ranges of the colonies. The distribution of mounds, however, suggested that these would have been less extensive than that of the *C. lacteus* colony described above.

The number of galleries intercepted by the encircling trench in the case of each mound was as follows (the number persisting at a distance of 6 feet from the centre of the mound being given in brackets):—*Mound A*, 27 (28); *Mound B*, 23 (18); *Mound C*, 27 (22); *Mound D*, 47 (36); *Mound E*, 30 (28). In four out of five mounds, the number of galleries persisting at 6 feet had decreased because more galleries joined than branched in the region of the trench, and also in several mounds one or two galleries ended blindly, having been blocked up, presumably, when they fell into disuse.

In the main, the distribution of galleries at the 5-ft. circle was well correlated with the distribution of food around the mound. Thus food was well distributed around Mound D, which had the greatest number of galleries, was less evenly distributed around Mound E, and was very unevenly distributed around Mounds B and C. In the case of the last-mentioned mounds, over two-thirds of the total number of galleries cut by the trench were found on the side (180° sector) where the greater part of the food was lying.

The galleries of *E. exitiosus* were found to run at about the same depth below the surface as did those of *C. lacteus*, and to evince the same tendency to tunnel below any gravel layer encountered. Thus, at the 5-ft. trench, all the galleries from Mounds A and E were between 3 and 9 inches from the surface. Mound D had a few galleries at a depth of 12 inches or over, Mound B rather more. Mound C was located in gravelly soil, and 13 of the 27 galleries radiating from it were over 12 inches below the surface at the trench, four being 18 inches or more deep. Two or three of the galleries penetrated the gravel, the remainder avoided it, when encountered, by passing below it.

The galleries of *E. exitiosus* are very different in appearance from those of *C. lacteus*. They are lined with a layer of blackish-brown carton material; and they tend to be oval in cross section, or alternatively to have a flat floor and a domed roof, the height being usually at least one-half of the width. Very few of the galleries travelling through the soil were more than $\frac{1}{2}$ inch wide; but when excavated in the wood of an old root they increased in size, often to 1 inch or more in diameter, and tended to become circular in cross section. They showed a much greater tendency to follow roots than did the galleries of *C. lacteus*, as will be seen by comparing the two plans.

Certain of the galleries of one of the mounds investigated were traced out from the trench until they connected with food; and these galleries were mapped by the method adopted in the *C. lacteus* study, the resulting plan being reproduced below (see Fig. 1). It will be seen at once that there is a difference in what may be called the general pattern of the galleries in the two species. In *C. lacteus* each gallery and its branches tend to remain separate, although more than one gallery may serve the same food mass. In *E. exitiosus* there are frequent cross-connexions between the galleries; and the gallery-pattern could be described as a reticulation rather than a ramification of a number of separate trunks.

5. Acknowledgment.

This investigation entailed a considerable amount of manual labour, which was lightened by the patience and enthusiasm of our assistants, Messrs. D. Banyard, G. Wearne, and A. Wetherly, whose help is here gratefully acknowledged.

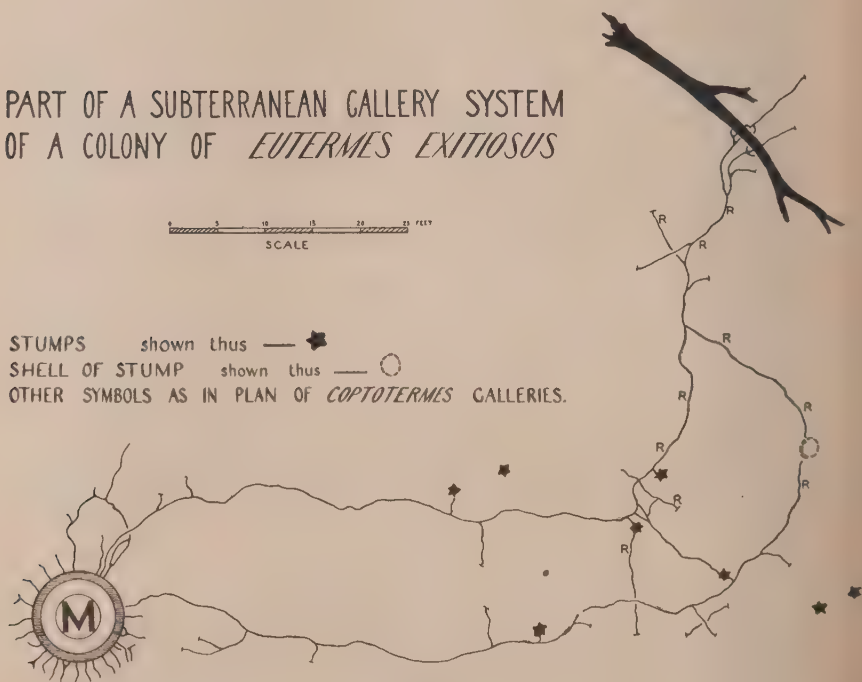

FIG. 1

THE SUBTERRANEAN GA
COPTOTER

Indicates		GALLERY WAS NOT TRACED FURTHER
	"	GALLERY WAS ·5 IN. WIDE
	"	GALLERIES CROSSED WITHOUT CONNECTION
SC	"	SHAFT CONNECTION BETWEEN GALLERIES
S	"	SHAFT TO SUBSOIL
R	"	GALLERY FOLLOWED ROOT
L	"	GALLERY WAS LOST

C.4558/40.

PLATE 1.

(Population of a Mound Colony of *Coptotermes lacteus* (Frogg.). See page 145.)

FIG. 1 (above).—Mound (No. 1, see text) of *Coptotermes lacteus* used in the population study. The overall length of the shovel shown in the photograph is 3ft. 3in.

FIG. 2 (below).—Same mound as in Fig. 1, with the outer clay envelope removed, photographed from a different angle and at closer range.

PLATE 2.

(The Subterranean Foraging Galleries of *Coptotermes lacteus* (Frogg.). See page 150.)

FIG. 1 (above).—Lightly timbered grazing land, with scattered logs and fallen trees, typical of the area in which the main gallery study was carried out. A mound of *C. lacteus* can be seen in the middle distance, behind a large grass tussock.

FIG. 2 (below).—The main trunk of a large foraging gallery of *C. lacteus* (No. 1, see plan) running six inches below the surface of the soil.

PLATE 3.

(A Survey of the Problem of Hoary Cress (*Lepidium draba* L.) as a weed in Victoria. See page 107.)

FIG 1.—Heavy sodium arsenite dressing followed by 3 cwt. per acre Weedex.

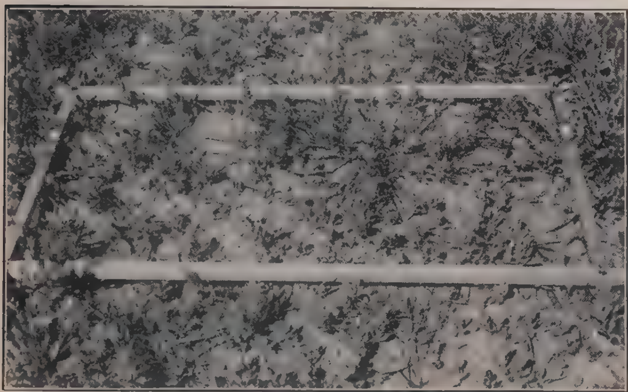

FIG. 2.—118 gals. carbon bi-sulphide per acre 1928.

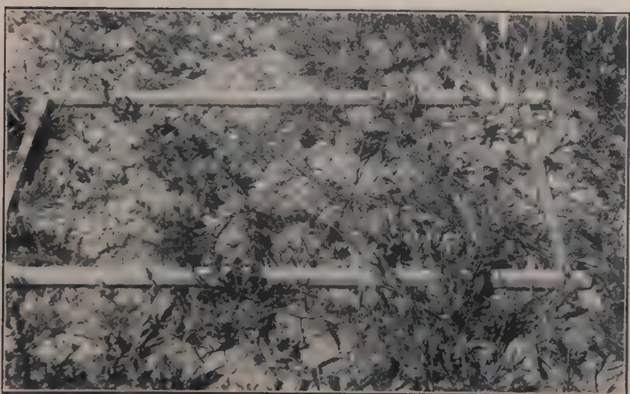

FIG. 3.—6 tons salt per acre 1926–1931.

PLATE 4.

(A Survey of the Problem of Hoary Cress (*Lepidium draba* L.) as a weed in Victoria. See page 107.)

Fig. 1.—Cultivated weekly 1927–28 and fortnightly 1928–30.

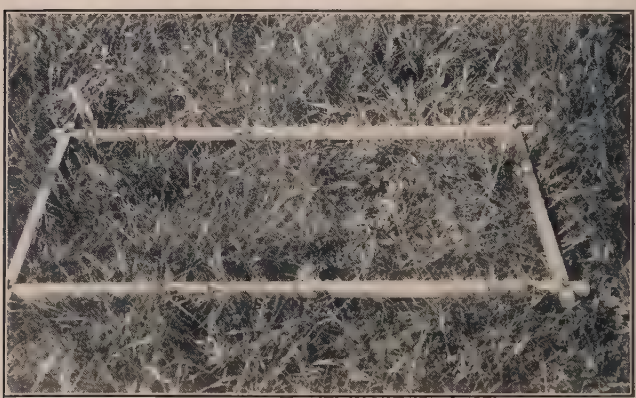

Fig. 2.—No treatment 1927–1939.

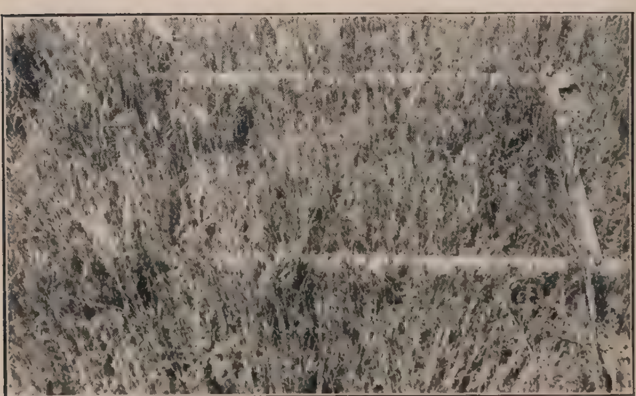

Fig. 3.—Sulphuric acid and thatch 1926, with regular acid sprays 1927–1931.

NOTES.

Fishery Explorations—Recent Results (March, 1940).

Nearly two years ago the *Warreen*—the fisheries research vessel of the Council for Scientific and Industrial Research—commenced her work of exploration of the possibilities of the pelagic fisheries (i.e., fisheries for fish like pilchard, tuna, salmon, &c., which swim in the upper water layers). The intervening period to date has been employed in scouring the waters between southern Queensland and South Australia for signs of such fishes, in order to obtain a fair idea of their regional and seasonal distribution. Provisional conclusions have now been formed in this connexion, and the vessel's activities are being increasingly directed to determining the best methods of catching these fish in commercial quantities.

It is probably not generally realized that for each type of fish the fishing technique is specialized. That is to say, it was impossible, at the outset, to rig the ship to catch commercial quantities of each and every type of fish (e.g., pilchard, tuna, salmon) which might possibly be encountered. It was merely possible to obtain, by various means, sufficient samples to determine the areas in which the various fishes occur.

The vessel's equipment is now gradually being altered so as to concentrate activities for the time being on the capture of two of the most abundant types of pelagic fish—tuna (of which there are several species, not all to be caught by the same type of gear) and pilchards.

Tuna.

Until recently, Tuna have been captured (often up to 2 tons a day) by the simple method of trolling behind the ship several feather or bone jigs. This method has since been used successfully at Eden by commercial fishermen using very small boats—such boats, during the season, being capable of catching up to nearly 200 8-lb. fish each per day's outing nearby. That is to say, the method is a payable one for small boats with a limited range, but the season for such boats is necessarily limited to a few months while the fish are running in the immediate vicinity. As canneries are springing up here and there on the south-east coast, it is expected that this type of small boat fishery for tuna will increase.

However, in order to obtain large catches and to extend the catching season over most of the year, larger vessels of a minimum size of from 55 to 65 feet are necessary, and the Fisheries Section of the Council for Scientific and Industrial Research is preparing blue prints and specifications of such vessels for issue to prospective builders. These vessels, while capable of using the Danish seine net for fishing during the most productive season for bottom fish such as flathead, will have provision for the establishment of a live-bait-carrying tank on deck, in which bait fishes can be kept alive and subsequently used for "chumming" tuna to the ship's side, where they can be caught by baited jig or by feather jig attached by wire traces to strong bamboo fishing poles.

Much significance attaches to the results of recent experiments of the *Warreen* which have demonstrated that at least one of the tuna species—the striped tuna or skipjack—can easily be caught by the live-bait method. This method was successfully employed on three occasions between 1st and 7th February in the region between Eden and Gabo. The live-bait used was the anchovy—taken by three methods (shore seining, ring netting, and mere scooping of fish attracted by submarine light) in Twofold Bay. The ring net, for example, encircled and caught a ton of anchovy at a setting.

Tuna clippers in California carry about 2,000 scoopfuls of live-bait. Having filled the *Warreen's* small bait tank (65 scoop capacity only) in which sea water is continuously circulated by pump, a search was made for one of the many tuna shoals in the vicinity. A few live anchovy were thrown out here and there until several tuna broke surface to seize them, whereupon several of the crew took up stations round the rail with poles and jigs. After several minutes, further numbers of anchovies having been cast overboard, the tuna came alongside in vast numbers, eagerly seizing the bait and such jigs as were baited with anchovy. The fishing tempo gradually increased as the tuna became more numerous and more excited and ceased to differentiate between baited jigs and feather jigs; fishing was then continued with feather jigs only, though live bait in reduced quantities was still thrown out. Within an hour 74 8- or 10-pound tuna were caught, and fishing was suspended when the limited supply of bait was expended. By this time catching was in full swing in a rough sea, every other cast resulting in a fish being hooked. It is considered that with a properly rigged vessel 5 tons could have been taken by this method from this small shoal alone in two or three hours' fishing. The research vessel is now being rigged with the requisite outboard platforms necessary for this type of fishing. The localities and seasons in which bait fishes occur are also being studied. Not all small fish are good bait for tuna. Though anchovy have been successful and live well in tanks, hardyheads and yellowtail have not been successful, as they dive when liberated, whereas anchovy remain at the surface. Pilchards and sprats have not yet been tried.

PILCHARDS.

A second important finding of recent weeks should be mentioned. Periodical searches for pilchards are made on the east coast, where the season has hitherto been regarded as being virtually limited to the winter months. During a summer cruise between 9th and 19th January, however, the *Warreen* located a succession of shoals between Newcastle and Broughton Island, and samples of these fish proved to be in fat condition (yielding 35 gallons of oil to the ton), as compared with the much leaner pilchard (1 to 5 gallons of oil to the ton) which have been caught in the winter months. It is possible that a pilchard fishery when developed would extend over a large portion of the year, and that the fish would be taken in better condition in the warmer than in the colder months of the year, when spawning occurs and depletes the fat reserves of the fish.

In the case of pilchards, as in that of tuna, the technique of catching on a large commercial scale is now being given more attention. The original net secured for pilchards has proved to be of too large a mesh, since the average size of the Australian pilchard, as far as results yet show, is much less than that of the Californian fish, and is able to pass through the meshes. The purse-seine net is, therefore, to be reconstructed with smaller mesh webbing, and meanwhile the method of gill-netting (drift-netting) is being developed, the small drift nets used for experimental purposes having three sizes of mesh so gauged as to catch fish of large size (if they prove to be present) or of the usual smaller sizes.

Obituary: Mr. A. L. Tonnoir.

By the sudden death, on January 27, of Mr. A. L. Tonnoir, the Division of Economic Entomology has suffered an irreparable loss. This loss is shared by the many entomologists throughout the Commonwealth and overseas who have, from time to time, benefited by his unusually wide knowledge and experience.

André Leon Tonnoir was born in Brussels in 1885. After his formal education at school and Liége University, he spent several years travelling in England, France, Germany, Italy, and Spain. During this period he was able to give much of his time to his outstanding interest, entomology, and, incidentally, to acquire a sound knowledge of six languages. However, it was not until after the Great War, when he was appointed to the staff of the Brussels Natural History Museum, that he was able to devote his whole time to his former hobby. Like many naturalists, he was especially interested in Australia and New Zealand, and in 1921 he accepted a commission to study the dipterous insects of the temperate zone of the Southern Hemisphere, a study which still occupied his leisure hours up to the day of his death.

He went to New Zealand, where he worked at the Cawthron Institute, the Canterbury Museum, and the Canterbury University College, until 1929, when he joined the staff of the Council in Canberra. During the last ten years he has been closely associated with research on biological control of insect pests and weeds, and he also played a prominent part in the development of an intensive study of the grasshoppers in Australia. Mr. Tonnoir's numerous papers on lesser-known families of the Diptera do not adequately reflect his remarkably wide knowledge of insects, although they are a record of the thoroughness of his work and his outstanding ability as a taxonomist.

His death removes a colourful personality and a delightful colleague, but our sadness is tempered by the knowledge that he died as he would have wished, peacefully in his sleep as he rested in the shade of a tree, after a morning's collecting in the bush.

Industrial Chemical Research—A New Laboratory.

In a previous issue (Vol. 11, p. 280) reference was made to the Commonwealth Government's approval of the recommendations of the Secondary Industries Testing and Research Committee that a National Standards Laboratory be established, that aircraft engine and testing research work be initiated, that a research service be established to carry out investigations of value to the secondary industries, and that the Council's existing information service be extended so that it would be better equipped to obtain and disseminate information regarding scientific and technological matters. Effect has already been given to three of these recommendations, namely, those concerning standards, aeronautical research, and information. The Government has recently provided the necessary funds to enable the Council to give effect to the fourth recommendation, namely, that concerning investigations of value to the secondary industries.

With that end in view, the sum of £50,000 has been made available to the Council for the erection and equipment of a laboratory alongside the Aeronautical Research Laboratory at Fishermen's Bend, Melbourne. Plans for the laboratory are not yet complete, but work on them is now in hand by the senior members of the new Division of Industrial Chemistry (Dr. I. W. Wark, who will have charge of the Division, and Mr. E. J. Drake). Before his appointment, Dr. Wark was for many years engaged on fundamental research work for the Electrolytic Zinc Company of Australia. He was accommodated in the Chemistry School of the University of Melbourne, where he established an international reputation for his work in the fields of flotation and surface chemistry. Mr. Drake, who was appointed to the staff of the Council's Information Section in 1938, was for many years a chemical consultant in Sydney.

The chief objectives of the new Division are five in number, namely, (i) to promote technical efficiency in industry, (ii) to stimulate new industries, (iii) to encourage the utilization of Australian raw materials, (iv) to encourage the replacement of imported materials by materials of Australian origin, and (v) to investigate the possibilities of industrial by-products. As a long-term policy, the Division will endeavour to play its part in bringing about better balance than the existing one between Australian primary and secondary industries.

It has been decided to organize the laboratory into five main sections, each in charge of a senior investigator. The sections in question are—(i) physical chemistry, (ii) organic chemistry, (iii) inorganic chemistry, (iv) analytical chemistry, and (v) chemical engineering. In other words, the laboratory will not be organized on a problem basis, but work on any particular problem will be undertaken by one or more of the above sections, each section looking into special aspects. Biochemical work may also be undertaken.

In the early years, work will be concentrated on five or six lines of study which seem to call for attention. These are mentioned in the following paragraphs:—

(i) *Non-metallic Minerals and their Utilization.*—In this field of work, early investigations will concern the beneficiation of minerals by concentration or flotation. Work on ceramics and cements would probably also be given attention.

(ii) *Metals and Alloys.*—An outstanding problem in this field is the corrosion of metals both under and above ground. The problem is a world-wide one, but aspects of peculiar Australian interest will probably be investigated.

(iii) *Wool and Other Fibres.*—Australia being an outstanding world producer of wool, it is believed that much useful work can be undertaken in connexion with wool scouring, and in the utilization of the wool wax and other by-products of the scouring industry. At the present time these by-products are sometimes wasted. Shrinkage of wool is another problem, and there also seems to be a field of work in connexion with heat and power utilization in textile mills.

(iv) *Hides and Leather.*—One problem in this field concerns the development of testing methods for the boot and shoe trade; another concerns the production of vegetable tanning materials of Australian origin. The fellmongery industry, too, is another promising field of work as the industry still depends very largely on the rule of thumb methods of olden times.

(v) *Dairy Products.*—There is little doubt that at the present time the butter industry of the world is seriously menaced by margarine. Accordingly, it behoves the dairying industry to put its house in order if it is to have any chance of meeting the competition. A programme relating to dairy products and, in particular, to the more efficient use in Australia of the different products of milk, has accordingly been formulated. It is hoped that this will be one of the first to be put in hand.

The programme for milk includes studies on drying and evaporation, on processed cheese, by-products utilization, cleaning, pasteurizing and cooling, churning in inert atmospheres, corrosion of tin surfaces, and sweetened condensed milk.

(vi) *Miscellaneous.*—Under this heading, it is hoped to give early attention to such matters as the peculiarly Australian problems relative to the production of paints and varnishes.

The Late Mr. S. Garthside—Grant from Hero Fund.

About twelve months ago the Council's Division of Economic Entomology suffered a loss on account of the death by drowning of one of its officers (Mr. S. Garthside), who had been sent to Great Britain some years previously to specialize on the collection and forwarding of insects likely to be of use in the control of Australian weed and insect pests.

Judging from recent press reports, the trustees of the Carnegie Hero Fund have awarded a memorial certificate and a weekly allowance of £2 to Mrs. Garthside, of Buckinghamshire, "whose husband—an Australian Government entomologist—was drowned while attempting to rescue a bather at Cornwall."

A New Casein Spray for Butter Boxes.

It is well known that, consequent on the restricted supply of New Zealand white pine for butter boxes, considerable difficulties were encountered from the use of Australian hoop pine. This timber, unfortunately, caused definite tainting of the butter, and materially

lowered its value on the export market. Consideration was given to the problem by the Council, and a method was eventually evolved to overcome the difficulty (see this *Journal* **5**: 15-24, 1932). The method consists essentially of spraying the inside surfaces of the butter boxes with a special casein borax solution and formaldehyde. This mixture when dry gives the surface of the wood a varnished appearance, and effectively seals, in the wood, in the butter taint producing materials. Its use has materially assisted the Australian dairy industry.

On account of the outbreak of war it appeared that there was a possibility of a shortage of supplies of formaldehyde, and accordingly consideration was given to the possibility of developing a new formula which would not necessitate the use of this chemical. In this regard some experimental work was carried out by Dr. W. J. Wiley, Dairy Research Officer of the Council, in co-operation with Mr. Wigan, Supervisor of Dairy Exports, and his officers in the Department of Commerce, with the Wirebound Box Co. Pty. Ltd., and with the Downs Co-operative Dairy Co., Queensland.

The casein formula used was as follows:—

Casein	100	parts	by	weight.
Water	400	„	„	„
Flake caustic soda	7	„	„	„
Commercial hydrated lime ..	20	„	„	„

The casein (40 mesh) was mixed with about three-quarters of the water and allowed to soak for 10 minutes. The caustic soda was dissolved in the remainder of the water, the lime suspended in this, and the whole added to the casein-water mix. This casein solution was more viscous than the usual casein-borax solution, and did not atomize so well. The first series of boxes sprayed, using a single spray gun, showed a definite thinning of the coating at the edge of the box shook. Tests were made with spray guns mounted in pairs, and these gave a satisfactory distribution of the casein solution. For test purposes some of the boxes, which had been coated using the single spray gun, were recoated with the double spray gun. The actual usable life of the casein-lime solution was 2¼ hours, but under commercial conditions it is considered that a working life of one hour should be allowed. It was noted also that the lime-casein coating, although it softens in contact with water (as do films of the usual casein-formalin type), does not become sticky and wet paper does not adhere to it. The coating on the wood soaks in somewhat more than when using the casein-formaldehyde type, and does not give such a finished appearance unless the quantity used is appreciably increased. The coating is odourless and tasteless a few days after application.

A total of 75 boxes were sprayed, and all these filled with butter of grade 94 points. One-half of the boxes representing the different types of spraying treatment were shipped to London, and the remainder placed in cold storage in Brisbane.

An examination of the boxes in Brisbane was made 11, 16, and 17 weeks after packing. The results of the three examinations were similar, in that any wood taint which might be present in the boxes which had been given a uniform coating (applied with two spray guns) was negligible. Boxes given the uneven coating using a single spray gun showed slight wood taint.

The London inspection, fourteen weeks after packing, was not quite so satisfactory, the report indicating that there was definite evidence of wood taint on the edges and surfaces of the butter, although beneath the

surface the butter was very good, grading 94 points. It is of importance to note that the boxes sprayed unevenly were less satisfactory than those given an even or a double coating.

The results of the experiment demonstrate that the new casein-lime formula is sufficiently satisfactory to replace the casein-formalin formula if the necessity should arise. The casein-lime solution is somewhat more difficult to apply, and is not appreciably cheaper to prepare, and its use is not advocated unless such is essential.

Recent Publications of the Council.

Since the last issue of this Journal, the following publications of the Council have been issued:—

Bulletin No. 131.—"Black End and Anthracnose of the Banana with Special Reference to *Gloeosporium musarum* Cke. and Mass.", by J. H. Simmonds, M.Sc., and R. S. Mitchell, M.Sc.Agr.

The investigations discussed in this publication were carried out by the Council in co-operation with the Queensland Department of Agriculture and Stock (of which Department one of the authors—Mr. J. H. Simmonds—is an officer), and were aided by grants made available by the Commonwealth Banana Committee, a body administering funds provided by the Government from duties collected on imported bananas. Infection with black end is shown to occur while the fruit is still in the plantation, although the disease does not become apparent until the fruit is ripened. It is concluded that control could only be obtained by careful attention to various points of plantation management, transport, and ripening. On the plantation, dead leaves on the plants proved to be the most important source of infection. Fruit should be handled carefully to avoid bruises and wounds through which rot-producing fungi might enter, and special care is necessary to prevent injury when breaking hands into singles. The general adoption of properly equipped ripening rooms is desirable.

Bulletin No. 132.—"The Wood Anatomy of Some Australian Lauraceae, with Methods for their Identification," by H. E. Dadswell, M.Sc., and Audrey M. Eckersley, M.Sc.

This Bulletin is the fourth of the series dealing with the anatomical features of the main families of Australian timbers. It deals with 31 different Australian species of the family Lauraceae, and, in addition, gives details of the habit and distribution of the tree and of the general properties of the timber. Photomicrographs showing details of the structure of the species have been included, as has a key for their identification.

Pamphlet No. 95.—"A guide to Picking for Export or Local Storage and to the Best Shipping Periods for Export Varieties," compiled by W. M. Carne (Fresh Fruit Officer, Department of Commerce, but formerly an officer of the Division of Plant Industry, C.S.I.R.).

In this Pamphlet, reports by Government officers on the condition of Australian apples in the United Kingdom markets, and experiments on the storage properties of certain varieties in Tasmania have been reviewed. It is concluded that a better out-turn of exported or locally stored fruit would result if the apples were picked within more restricted

periods; if the fruit to be cool-stored or exported is in most cases cooled within seven days of picking; if the export of any variety is confined to a limited period; and if reasonable conditions of storage are available. Suitable dates before which varieties should not be picked are given. The Pamphlet is the second of the Council's publications printed by the photolithographic process, which has been introduced as a war time economy measure.

"*Handbook of Structural Timber Design.*"—This Handbook, for which the Division of Forest Products has been responsible, contains a large mass of data which will help engineers and architects to use Australian structural timbers in the most efficient way; it will be of particular value in the present time of war in showing how best to replace by an Australian product the present large imports of timber, much of which comes from non-sterling countries. The publication contains some 283 pages, and largely consists of tables of strengths of different sized sections and lengths of different Australian woods. The cost of printing was considerable, but to meet it in part, various interested bodies such as Timber Development Associations, Forest Services, Institution of Engineers (Australia) and various timber organizations made contributions totalling nearly £600. The book is of no interest to people having no knowledge of the design of structures.

Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

Bulletin No. 133.—"A Soil Survey of the Mildura Irrigation Settlement, Victoria," by F. Penman, M.Sc., G. D. Hubble, B.Agr.Sc., J. K. Taylor, B.A., M.Sc., and P. D. Hooper.

Bulletin No. .—"Investigations on the Storage of Jonathan Apples grown in Victoria," by S. A. Trout, M.Sc., Ph.D., G. B. Tindale, B.Sc.Agr., and F. E. Huelin, B.Sc., Ph.D.

Pamphlet No. 96.—"Further Investigations on Copper Deficiency in Plants in South Australia," by D. S. Riceman, B.Ag.Sc., C. M. Donald, B.Sc.Agr., and S. T. Evans, B.Sc.

Pamphlet No. 97.—"The Shrinkage of Australian Timbers. 2.—Shrinkage Data for 170 Timbers," by W. L. Greenhill, M.E., Dip.Sc.

Pamphlet No. 98.—"Studies on Chemical Weed-killers, with Special Reference to Skeleton Weed," by C. G. Greenham, M.Sc., G. A. Currie, D.Sc., B.Agr.Sc., and F. E. Allan, M.A.

Pamphlet No. .—"Studies on the Marketing of Fresh Fish in Australia. Part 2.—The Bacteriology of Spoilage of Marine Fish," by E. J. Ferguson Wood, M.Sc., B.A., A.A.C.I.

Pamphlet No. .—"Studies of the Physiology and Toxicology of Blowflies. 2.—The Action of Stomach Larvicides of *Lucilia cuprina,*" by F. G. Lennox, M.Sc., A.I.C. 3.—"The Toxicity of Some Arsenicals to Larvae of *Lucilia cuprina,*" by F. G. Lennox, M.Sc., A.I.C., and L. G. Webber. 4.—"The Action of Contact Larvicides on *Lucilia cuprina,*" by F. G. Lennox, M.Sc., A.I.C.

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